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AN INVESTIGATION OF PHOSPHORUS REMOVAL MECHANISMS IN ACTIVATED SLUDGE SYSTEMS

W. E. Morgan, et al

National Environmental Research Center
Cincinnati, Ohio

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16. Abstract The magnitude of two phosphorus removal mechanisms, metabolic uptake and chemical precipitation with calcium, in activated sludge systems were investigated using synthetic substrates representative of actual wastewaters. Using completely mixed continuous flow laboratory activated sludge units with operating conditions that precluded significant precipitation of phosphorus, normal growth defined as constant 85 to 90 percent carbon removal occurred above 0.9 to 1.0 percent sludge phosphorus (influent COD:P ratio of 670:1). Between 1.0 and 1.6 percent (influent COD:P ratio of 220:1) a storage zone existed with all phosphorus present utilized, and above 1.6 percent a variable saturation zone occurred with an upper limit near 3.0 percent. An alkaline phosphatase bioassay verified qualitatively the normal growth phosphorus requirement and storage zone, but did not define the upper limit of the saturation zone. An acclimated activated sludge unit with a substrate containing 2 mM calcium, 0.4 mM phosphorus, 0.8 mM magnesium and 2.5 mM bicarbonate attained a maximum of 3.7 percent sludge phosphorus after 39 days of operation at pH 7.6. A similar system with the addition of 1 mg/l fluoride attained 4.6 percent sludge phosphorus. An increase in magnesium to 2.0 mM had little effect on phosphorus precipitation. Alkalinity was implicated to exert both a kinetic effect as well as an effect on residual soluble phosphorus in calcium-phosphorus systems. The presence of soluble organics also was shown to be inhibiting with increasing concentrations. ~~(Morgan~~

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AN INVESTIGATION OF PHOSPHORUS REMOVAL MECHANISMS IN ACTIVATED SLUDGE SYSTEMS

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ABSTRACT

The magnitude of two phosphorus removal mechanisms, metabolic uptake and chemical precipitation with calcium, in activated sludge systems was investigated using synthetic substrates representative of actual wastewaters. Using completely mixed continuous flow laboratory activated sludge units with operating conditions that precluded significant precipitation of phosphorus, normal growth defined as constant 85 to 90 percent carbon removal occurred above 0.9 to 1.0 percent sludge phosphorus (influent COD:P ratio of 670:1). Between 1.0 and 1.6 percent (influent COD:P ratio of 220:1) a storage zone existed with all phosphorus present utilized, and above 1.6 percent a variable saturation zone occurred with an upper limit near 3.0 percent.

An alkaline phosphatase bioassay verified qualitatively the normal growth phosphorus requirement and storage zone, but did not define the upper limit of the saturation zone.

An acclimated activated sludge unit with a substrate containing 2 mM calcium, 0.4 mM phosphorus, 0.8 mM magnesium and 2.5 mM bicarbonate attained a maximum of 3.7 percent sludge phosphorus after 39 days of operation at pH 7.6. A similar system with the addition of 1 mg/l fluoride attained 4.6 percent sludge phosphorus. An increase in magnesium to 2.0 mM had little effect on phosphorus precipitation. Alkalinity was implicated to exert both a kinetic effect as well as an effect on residual soluble phosphorus in calcium-phosphorus systems. The presence of soluble organics also was shown to be inhibiting with increasing concentrations.

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SECTION I

CONCLUSIONS

1. The limits of metabolic incorporation of phosphorus in completely mixed continuous flow laboratory activated sludge systems are:
    a. the minimum required sludge phosphorus content for normal growth, i.e., a constant 85 to 90 percent carbon removal, is approximately 1 percent and occurs at an influent COD:phosphorus ratio of 670:1 (100:0.15);
    b. sludge phosphorus content greater than 1 percent represents "luxury uptake" and is the rule and not the exception in activated sludge systems;
    c. a storage zone exists between 1 and 1.6 percent sludge phosphorus content where all phosphorus present in the influent is utilized;
    d. all phosphorus in the influent is utilized and a sludge phosphorus content of 1.6 percent occurs with an influent COD:phosphorus ratio of 220:1 (100:0.45);
    e. an increase in influent phosphorus beyond the optimum influent COD:phosphorus ratio of 220:1 will result in an increase in sludge phosphorus content but not all the phosphorus present in the influent will be utilized; and,
    f. the maximum sludge phosphorus content obtained by metabolic incorporation is approximately 3 percent.

2. The minimum required, storage, and saturation zones for metabolic incorporation of phosphorus were qualitatively defined by the alkaline phosphatase bioassay; however,
    a. the bioassay did not define the upper limit of metabolic incorporation of phosphorus, and
    b. a quantitative relationship between alkaline phosphatase activity and percent sludge phosphorus content was not obtained, thereby precluding an engineering application as an operating tool.

3. Phosphorus incorporation in activated sludge greater than the maximum possible by metabolic mechanisms was obtained at pH 7.6 in six week acclimated completely mixed continuous flow laboratory activated sludge systems with precipitation of phosphorus restricted to calcium-phosphorus forms.

4. A sludge phosphorus content of 3.7 percent was obtained with calcium-phosphorus precipitation which is much lower than the sludge phosphorus contents reported, 6 to 7 percent, in activated sludge plants exhibiting high phosphorus removals, e.g. San Antonio Rilling, Baltimore.

5. High influent magnesium concentrations (Ca:Mg ratio $\sim$ 1) did not affect the sludge phosphorus content, but did delay the start of a calcium-phosphorus precipitation in an activated sludge system.

6. The addition of 1 mg/l fluoride increased the sludge phosphorus content to 4.6 percent removing over twice the phosphorus required for normal metabolic requirements.

7. Calcium-phosphorus systems are sensitive to upsets in operating pH conditions and the presence of fluoride did not affect a calcium-phosphorus system within 50 days after upset.

8. Other factors were found that affect calcium-phosphorus systems in activated sludge:
    a. organic concentration has a substantial negative effect on calcium-phosphorus precipitation;
    b. the normal events of high dissolved oxygen and increase in pH of plug flow systems can be described as symptoms of removal of an organic poisoning effect of calcium-phosphorus precipitates by microbial incorporation of biologically degradeable carbon; and,
    c. alkalinity affects dilute calcium-phosphorus systems and appears to exert a significant effect at high concentrations.

SECTION II

RECOMMENDATIONS

During the course of this laboratory investigation of metabolic incorporation of phosphorus and chemical precipitation of phosphorus with calcium, two areas with potential as phosphorus removal methods were noted and should be further investigated.

The storage zone defined for activated sludge, between 1 and 1.6 percent, exhibits an uptake of essentially all phosphorus present in the influent. Studies should be undertaken to evaluate techniques for rapid stripping of phosphorus from sludge to obtain the low sludge phosphorus contents required. The characteristics of such low phosphorus content sludges should be investigated, particularly of solids-liquid separation and organic uptake within existing system designs. If efficient operation within the storage zone could be attained, relatively low effluent phosphorus concentrations would be possible using a metabolic removal mechanism. Such a method could be appropriate for municipal wastewaters with below average influent phosphorus concentrations, less than 8 mg/l P.

Precipitation of phosphorus with calcium, though not sufficient to account for the high removals reported by some activated sludge plants, does indicate that a chemical removal mechanism can occur in activated sludge. Additional studies are indicated to investigate the presence of small quantities, less than 3 to 5 mg/l, of other cations such as Fe III and Al III that have been reported to be reduced in concentration passing through activated sludge plants. Separate and combined cation studies could identify the optimum cation(s) and concentration(s) to induce significant chemical removal of phosphorus.

Also, a possible organic poisoning effect on such chemical precipitation should be investigated. Such an effect could be present in some plants exhibiting marginal organic and poor phosphorus removal characteristics. Improved organic removal could possibly enhance phosphorus removal with no additional changes in a system.

SECTION III

PURPOSE AND NEED OF THE STUDY

The quantity of phosphorus incorporated by activated sludge has been reported by several investigators to range between 2 and 3 percent based on volatile suspended solids present in the sludge (1,2,3). While this range is considered "normal" and is found in most activated sludge systems, there have been some activated sludge systems reported to exhibit higher percentages of incorporated phosphorus, up to 7 percent in some cases (4,5). This unusual and unaccounted for incorporation of phosphorus has led to several investigations to determine the mechanism(s) responsible for this phenomenon.

Two hypotheses for this abnormally high sludge phosphorus are:

1) a biological incorporation in excess of required metabolic uptake by the biomass present in the sludge, so called "luxury uptake"; and
2) a physical-chemical formation of a solid specie that subsequently is sorbed or becomes enmeshed in the sludge mass.

These two viewpoints have caused considerable discussion in the literature and both hypotheses can be at least partially validated. However, insufficient field and laboratory data exist to substantiate clearly the mechanism(s) responsible for the high phosphorus content of some sludges.

Biological incorporation of phosphorus occurs with the synthesis of new cells, but the magnitude of phosphorus incorporation beyond that necessary for new growth is not well-defined. Some means of isolating biological uptake and evaluating the magnitude of this phosphorus removal mechanism quantitatively could aid in determining if other mechanism(s) are involved significantly in the removal of phosphorus from domestic wastewaters.

In this section a thorough evaluation of the literature is conducted, including research on the biological uptake of phosphorus, various chemical mechanisms of phosphorus removal and the field studies which have attempted to define the operating conditions that could have induced the high sludge phosphorus content observed at several

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activated sludge plants. Subsequently, the specific objectives and scope of this investigation are outlined.

Biological Removal of Phosphorus

A major phosphorus removal mechanism found in activated sludge systems is the biochemical uptake of phosphorus to satisfy the metabolic requirements of the biomass. For normal activated sludge systems which remove a small percentage of the influent phosphorus, the accepted range of cellular phosphorus content is between two and three percent of the volatile solids. However, several investigators (4,5) reported activated sludges with significantly higher sludge phosphorus contents, and the capability of removing much higher percentages of the influent phosphorus which they have attributed to "luxury uptake" of phosphorus (6,7,8). These differences have led to some discussion as to the amount of phosphorus that is metabolically required, or that can be assimilated by the biomass.

Metabolic Phosphorus Requirements

The phosphorus assimilation for specific microorganisms is variable. The presence of volatin granules reported to contain polyphosphate has been observed in some bacteria (9).

Varma and Stonefield (10) reported different phosphorus assimilation through a growth cycle for E. coli and P. vulgaris using P^{32} techniques. Subsequent tests also exhibited significant differences between these pure cultures and mixed activated sludge cultures. Yall, et al. (11) using P^{32} techniques reported the results of 88 bacterial isolates from an activated sludge exhibited variable phosphorus assimilation.

A discussion of the biological assimilation of phosphorus to fulfill the metabolic requirements of the mixed bacterial cultures in activated sludge systems should be prefaced by a short discussion of the parameters required to define this removal mechanism. A number of past studies (1,12,13,14) have reported required ratios of carbon, as biochemical oxygen demand (BOD), to phosphorus in the influent waste, to sustain efficient operation of activated sludge systems, i.e. BOD removal and rapid solid-liquid separation. The reported ratios of BOD to phosphorus, 100 to 1, satisfy these factors and preclude phosphorus from being a limiting nutrient in domestic wastewater treatment systems. However, these ratios do not define the limits of the metabolic uptake of phosphorus by bacteria, i.e. the minimum phosphorus required for normal growth and the maximum phosphorus that can be biologically incorporated. The metabolic uptake of phosphorus by bacteria should be defined within these limits.

Greenberg, et al. (15) investigated the minimum required sludge phosphorus content for good growth using "fill and draw" laboratory units. The substrate used had a BOD:P ratio of 100:0.42 and an ionic composition which precluded any significant phosphorus removal by chemical precipitation. They reported that BOD removals greater than 90 percent occurred when the sludge phosphorus content was one percent or greater, but below this value BOD removal declined. As they increased the phosphorus in the substrate (BOD:P ratios of 100:0.95 and 100:2.6), the sludge phosphorus content increased to a high of 1.8 percent.

Hattingh (16) also investigated the effect of low sludge phosphorus content using "fill and draw" laboratory units. During these experiments the sludge phosphorus content was reduced to less than 1.0 percent after 32 days with a substrate having a BOD:P ratio of 100:0.24, but the biomass still removed greater than 95 percent of the influent BOD. Using a substrate with a BOD:P ratio of 100:1.65 a maximum sludge phosphorus content of 2.2 percent was obtained. As with Greenberg's study, the composition of the substrates precluded any significant phosphorus removal by chemical precipitation.

Greenberg reported that the biomass in the low phosphorus cultures was primarily filamentous with poor solid-liquid separation characteristics. Hattingh reported no marked differences in protozoal or bacterial population between the high and low phosphorus cultures; however, some tendency to bulk occurred in the low phosphorus cultures. The low phosphorus content of the bacteria did not affect the BOD removal efficiency, but did affect the solid-liquid separation characteristics of the sludges.

Borchardt and Azad (17) conducted experiments to determine the lower limit of phosphorus content in algae to maintain good growth. Using cultures of Chlorella and Scenedesmus they reported three cellular phosphorus content regimes under steady state growth conditions: the storage zone, one to three percent cellular phosphorus; the growth dependent zone, zero to one percent cellular phosphorus; and the saturation zone, greater than three percent (see Figure 1.). Within the one to three percent cellular phosphorus regime, maximum growth occurred with the removal of all phosphorus from the substrate. Above

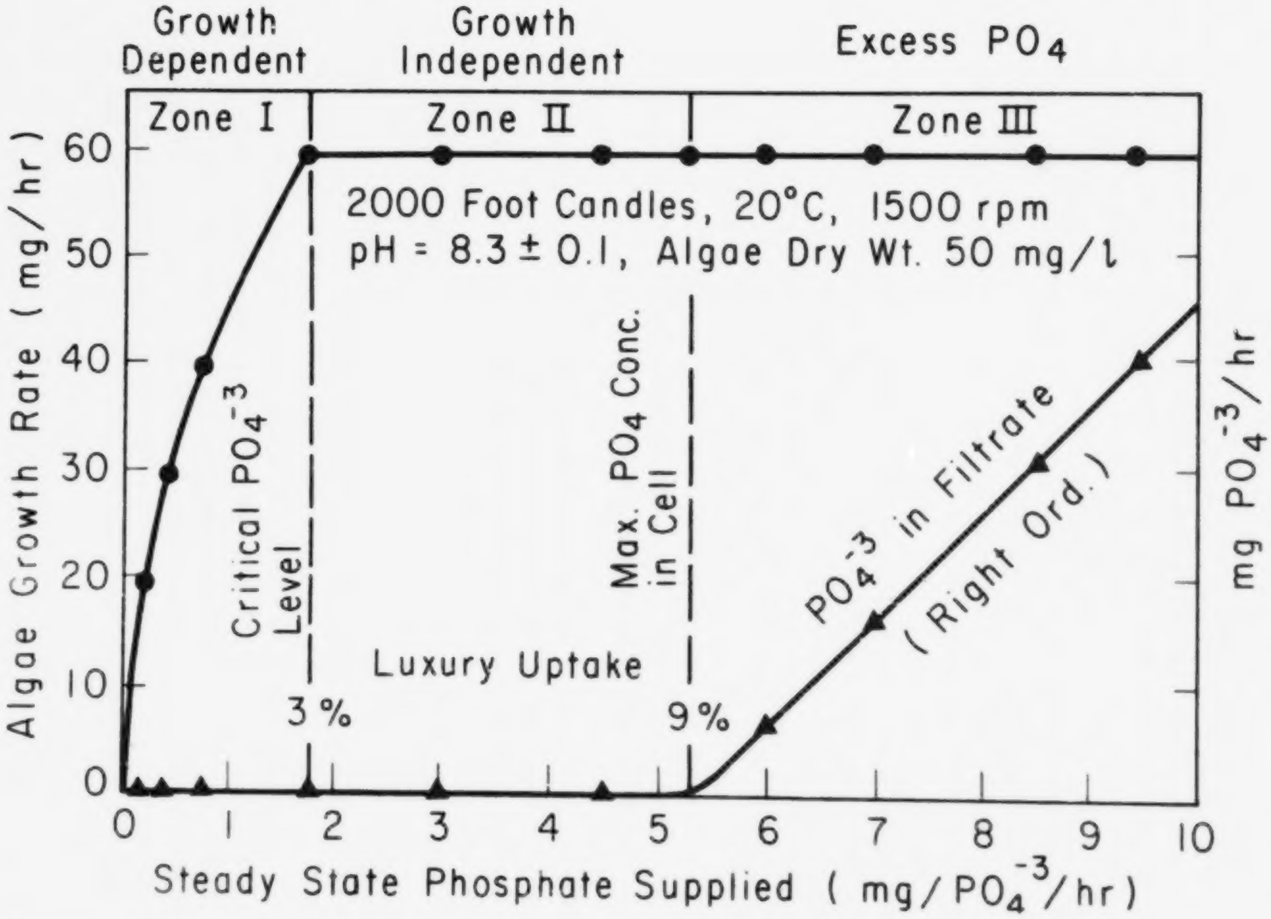

Figure 1. Zones of Sludge Phosphorus Content for Algae (after Borchardt and Azad).

three percent cellular phosphorus additional phosphorus in the substrate above that required to maintain the three percent cellular phosphorus level appeared in the effluent. Moore and Fruh (18) reported similar results with various algal cultures. The lower limit of cellular phosphorus content for maximum growth in these algal experiments was approximately one percent and agreed with the conclusions of Greenberg, et al. and Hattingh based on BOD removal by mixed cultures of activated sludge microorganisms.

Other investigations have been conducted to determine only the maximum cellular phosphorus content of activated sludge and the results have indicated that the upper limit of cellular phosphorus approaches three percent (19,20). A recent extensive study of sludge phosphorus content by Jenkins and Menar (3) covering a wide range of organic loading rates, 0.2 to 13.8 pounds of BOD per pound of volatile suspended solids, reported a minimum sludge phosphorus content of 2.45 percent and a maximum of 3.03 percent with a weighted mean of 2.62 percent.

There are instances reported in the literature that have alluded to "luxury uptake" occurring during laboratory tests. Randall, et al. (21) conducted tests to investigate phosphorus release by activated sludge. They observed that phosphorus uptake was remarkably high, an average of 9 mg/l P associated with approximately 250 mg/l COD removal. They implied that this high phosphorus removal suggested "luxury uptake." However, their substrate medium did not preclude phosphorus precipitation.

Following 9 mg/l P removal, the increase in the percent sludge phosphorus based on the original 2000 mg/l suspended solids was calculated from other data presented by Randall, et al. to be 0.45 percent. No data were available on the initial percent sludge phosphorus for comparison, but an increase of 0.45 percent would not be unusual in a batch test. Additionally, the difference between the phosphorus uptake and phosphorus subsequently released during the leaching phase of the experiments averaged 2.5 mg/l. If this is assumed to be incorporated in new growth (approximately 90 mg/l for 250 mg/l COD removed), the percent sludge phosphorus content was approximately 2.7 percent and not indicative of unusual phosphorus incorporation.

Wells (22) reported the results from several batch type experiments at the San Antonio Treatment Plants using raw wastewater and hence also did not preclude phosphorus precipitation. To show the effect of vigorous aeration on phosphorus uptake, samples from the return sludge of the Rilling, East and West Plants were mixed with the same sample of raw wastewater and aerated vigorously. The Rilling plant sludge rapidly removed 14 mg/l P in approximately one hour. The East Plant sludge removed approximately 2 mg/l P in eight hours, while the West Plant removed 12 mg/l P during the same eight hour aeration period. In similar previous aeration batch tests the East and West sludges had removed little phosphorus. Thus, Wells indicated the possibility of the West Plant sludge becoming somewhat more "active" in phosphorus removal.

Using the data reported, the initial phosphorus contents of the sludges were computed and are presented in Table 1. Though initially different, the final sludge phosphorus content of the East and West Plant sludges during the above test series are essentially the same, and are not indicative of unusual phosphorus removal. The Rilling plant sludge phosphorus content was initially high, 3.9 percent, and did increase to 4.35 percent. However, this sludge previously had been exhibiting such phosphorus removal characteristics and the mechanism responsible has not been determined. (The Rilling Plant's phosphorus removal is discussed further in subsequent sections of this review.)

Similar results have been reported for batch type experiments (8,23), but the unusual phosphorus removals or percent sludge phosphorus contents have been associated only with the lag phase of the growth cycle. This phase of the growth cycle normally is associated with the uptake of nutrients without a significant increase in the number of cells present. These phenomena can be observed by measuring this increase in cellular content of several nutrients, including phosphorus (24).

An Enzymatic Technique to Detect Surplus Phosphorus Uptake

The requirement for a quantitative measurement of phosphorus assimilated by the microorganisms is provided by the alkaline phosphatase enzymes utilizing p-nitrophenylphosphate as a sole source of phosphorus. This compound is colorless, but upon hydrolysis of the phosphate group the yellow salt, p-nitrophenol, is liberated. The reaction occurs as follows:

$$\underset{\text{(colorless)}}{\text{p-nitrophenylphosphate}} + H_2O \xrightarrow[35^{\circ}C]{\text{alkaline phosphatase}} \underset{\text{(yellow)}}{\text{p-nitrophenol}} + H_3PO_4$$

Table 1

Phosphorus Uptake by Activated Sludge at San Antonio, Texas
(after Wells)

	Initial Conditions					Final Conditions		
Plant	Total phosphorus mg/l	Soluble P mg/l	Sludge P mg/l	Suspended solids mg/l	Percent P	Soluble P mg/l	Sludge P mg/l	Percent * P
East	80	8	72	3290	2.2	6.0	74	2.25
West	60	12	48	2760	1.75	0.0	60	2.2
Rilling	128	14	114	2940	3.9	0.0	128	4.35

* No data supplied on final suspended solids; therefore, sludge phosphorus based on initial solids.

Thus, if p-nitrophenylphosphate is the sole source of phosphorus, the alkaline phosphatase activity can be directly related to the quantity of p-nitrophenol present. The rate of the reaction can be determined photometrically (absorption maximum, 410 mμ) measuring the increase in p-nitrophenol with time.

Fitzgerald and Nelson (25) developed an alkaline phosphatase bioassay technique for detecting surplus metabolic uptake of phosphorus by algae. In their work they found significant differences in the alkaline phosphatase activity of algae that were phosphorus deficient when compared to algae that were not phosphorus-limited.

Using the technique developed by Fitzgerald and Nelson with modifications reported by Fruh and Pessoney (26), Moore, et al. (23) evaluated the alkaline phosphatase bioassay to detect surplus phosphorus uptake by bacteria. Their experiments were conducted using batch units inoculated with mixed bacterial culture. During the experiments bacteria grown in phosphorus-limiting substrates and containing varying amounts of cellular phosphorus were placed in batch units containing different concentrations of phosphorus. At different phases in the growth cycle an alkaline phosphatase assay was conducted and phosphorus content of the sludge was measured. These investigators reported that there was an apparent correlation between the cellular phosphorus content and the enzymatic activity exhibited by the bacteria. Some of their results are presented in Figure 2. However, these results are based on batch unit tests and do not include all the variables possible in a continuously fed system, i.e., constant nutrient supply and relatively constant quantity of bacteria present. In addition, the high phosphorus sludge concentrations only were found in the lag phase of the growth cycle.

Chemical Removal of Phosphorus

Domestic wastewaters generally contain all of the cations necessary for the formation of insoluble forms of phosphorus. The concentrations of these cations are a function of the water supply, types of industrial wastes discharged into the system, and the characteristics of the soil associated with surface runoff and infiltration. Though phosphorus will combine with many species, the three aqueous metal ions Al III, Fe III and Ca^{++} are generally considered to be the primary cations involved in the formation of insoluble forms of phosphorus. Investigations at the Baltimore Back River Wastewater Treatment Plant (27) and the Los Angeles Hyperion Treatment Plant (28) have reported apparent balances of phosphorus removed with these and other cations.

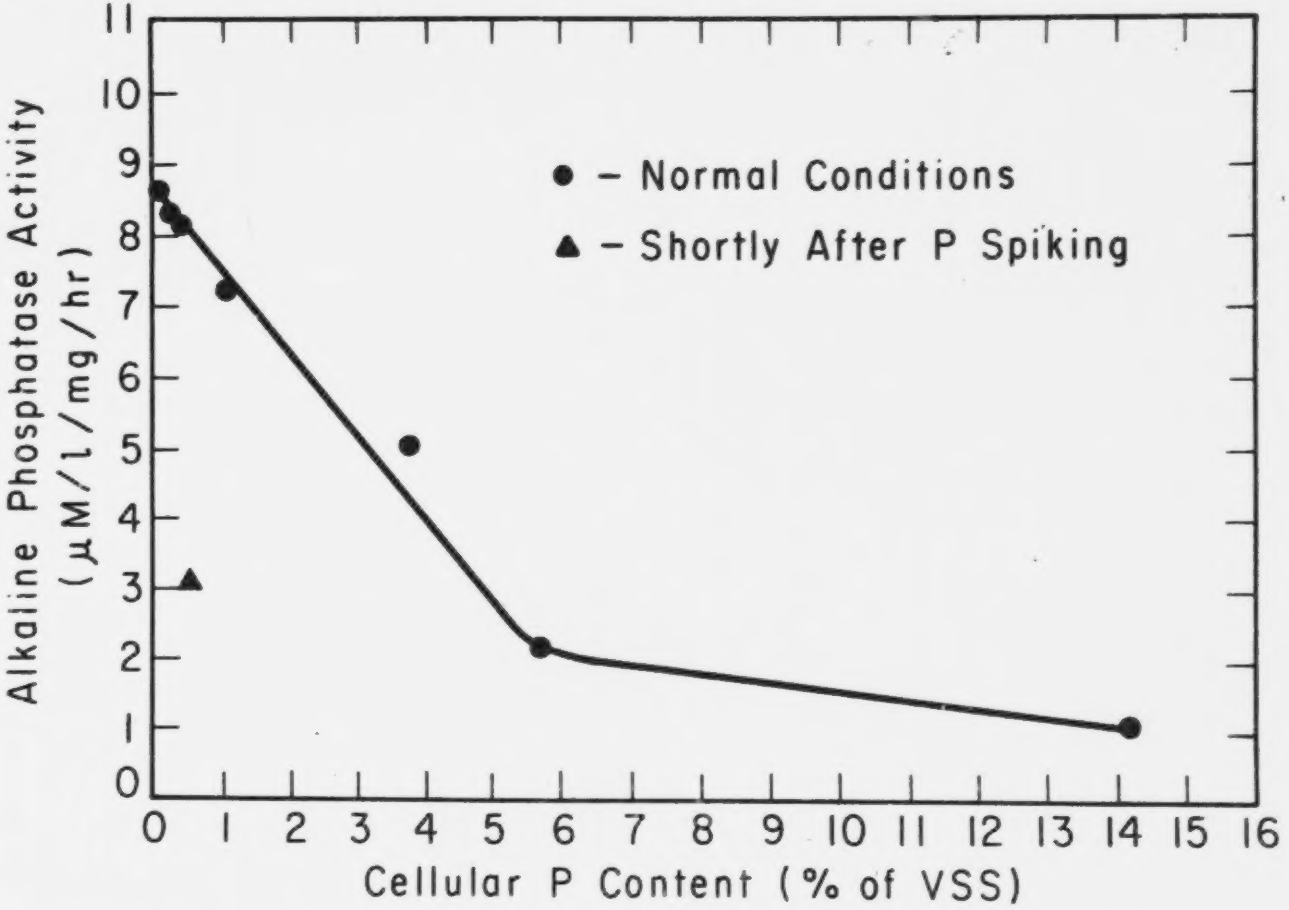

Figure 2. Relationship of Enzyme Activity and Cellular Phosphorus Content in Dilute Bacterial Cultures (after Moore, et al.).

In laboratory and field investigations the precipitation of inorganic phosphorus with Fe III and Al III generally conform to the solubility diagrams for $FePO_4$ and $AlPO_4$ (29,30,31,32). However, solubility predictions for precipitation of phosphorus with calcium are difficult to corroborate experimentally, particularly in short-term experiments (33). In general the concentrations of Fe III and Al III in domestic wastewaters are much smaller than calcium. If phosphorus removal by chemical precipitation is occurring naturally, i.e. no addition of chemical coagulant to the activated sludge, it would be reasonable to assume that the major cation involved would be calcium. This assumption was made in this research and chemical removal of phosphorus was limited to calcium-phosphorus systems.

In discussing solid forms of calcium-phosphorus systems three compounds have been considered the major forms of interest in wastewaters:

1) dibasic calcium phosphate, $CaHPO_4$;
2) the apatites; and
3) beta-tricalcium phosphate.

Other forms exist (see Appendix A), but are not considered to contribute significantly to removal of phosphorus from natural aquatic environments or wastewaters. This review of the literature on the solubility of calcium-phosphorus compounds is intended to present the possibilities that may occur in calcium-phosphorus systems, such as found in activated sludge systems, and not as a detailed study of these forms. Variables and parameters that can influence the formation of these solid forms, whether in the kinetics involved or in interference or enhancement from the presence of other species, have been noted to indicate the complex nature of the calcium-phosphorus system. Difficulties of corroboration using X-ray diffraction and infrared absorption techniques also are noted.

Dibasic Calcium Phosphate

Dibasic calcium phosphate has been postulated to occur in activated sludge systems, but little evidence supports this premise. Although the theoretical solubility of $CaHPO_4$ is exceeded in domestic wastewaters, the theoretical solubilities of the apatites and beta-tricalcium phosphate are more than an order of magnitude lower within the pH range encountered. Leckie and Stumm (33) could not confirm $CaHPO_4$ precipitation in their laboratory using solutions comparable to sewage (e.g. $[Ca^{++}] = 2 \times 10^{-3}$ M, $[HCO_3^-] = 10^{-3}$M, $P_{total} = 10^{-3}$M, pH= 7.7). The resulting precipitate, although poorly crystalized, gave X-ray patterns of hydroxyapatite.

Apatites

Apatites, $Ca_{10} (PO_4)_6 (X)_2$, are the least soluble of the calcium-phosphorus precipitates. Hydroxyapatite, $Ca_{10} (PO_4)_6 (OH)_2$, is representative of this group, has been studied extensively, and will be used to describe this group.

Hydroxyapatite has certain well-defined characteristics which can be identified by infrared absorption or X-ray diffraction. It theoretically has a Ca:P ratio of 1.67, but has been observed to form with ratios from 1.3 to 2.0. Controversy exists as to whether or not equilibrium is attained, but it is generally agreed that it is a slow process at normal temperatures (33). These properties have led to some confusion in determining the existence or nonexistence of apatites in activated sludge systems. The variable molar ratio and the inability to obtain X-ray or infrared verification of apatites in some activated sludge studies (34,35) have precluded definitive identification in these heterogeneous solutions.

Kinetics of Solid Formation: Ferguson and McCarty (35) investigated the formation of solid phases from calcium-phosphorus systems. They derived a theoretical model based on six components (total concentrations of Ca^{++}, $PO^{4\equiv}$, $CO_3^{=}$, Mg^{++}, H^+ and H_2O) to predict the type of solid phases precipitated (see Figure 3). In systems with no magnesium they predicted a minimum soluble phosphorus residual between pH 7 and 8 with calcium to phosphorus ratios greater than two. This low solubility was attributed to the precipitation of hydroxyapatite. Above pH 8 the soluble phosphorus increased due to competitive precipitation of calcite.

In the experimental tests they confirmed their model for the low magnesium system, but did not attain the low soluble phosphorus residuals predicted by the model. They obtained soluble phosphorus residuals between pH 7 and 8 in the 10^{-4} molar region and above pH 8 the residuals increased to 10^{-3} molar. The soluble phosphorus residual did not decrease until above pH 10. X-ray diffraction and infrared absorption measurements indicated the precipitates contained apatite. The time period of the tests was 24 hours.

These residuals are higher than predicted from theoretical solubility equilibria, but can be explained partially by the crystal forming kinetics of hydroxyapatite. Corsaro, et al. (36) investigated the formation of

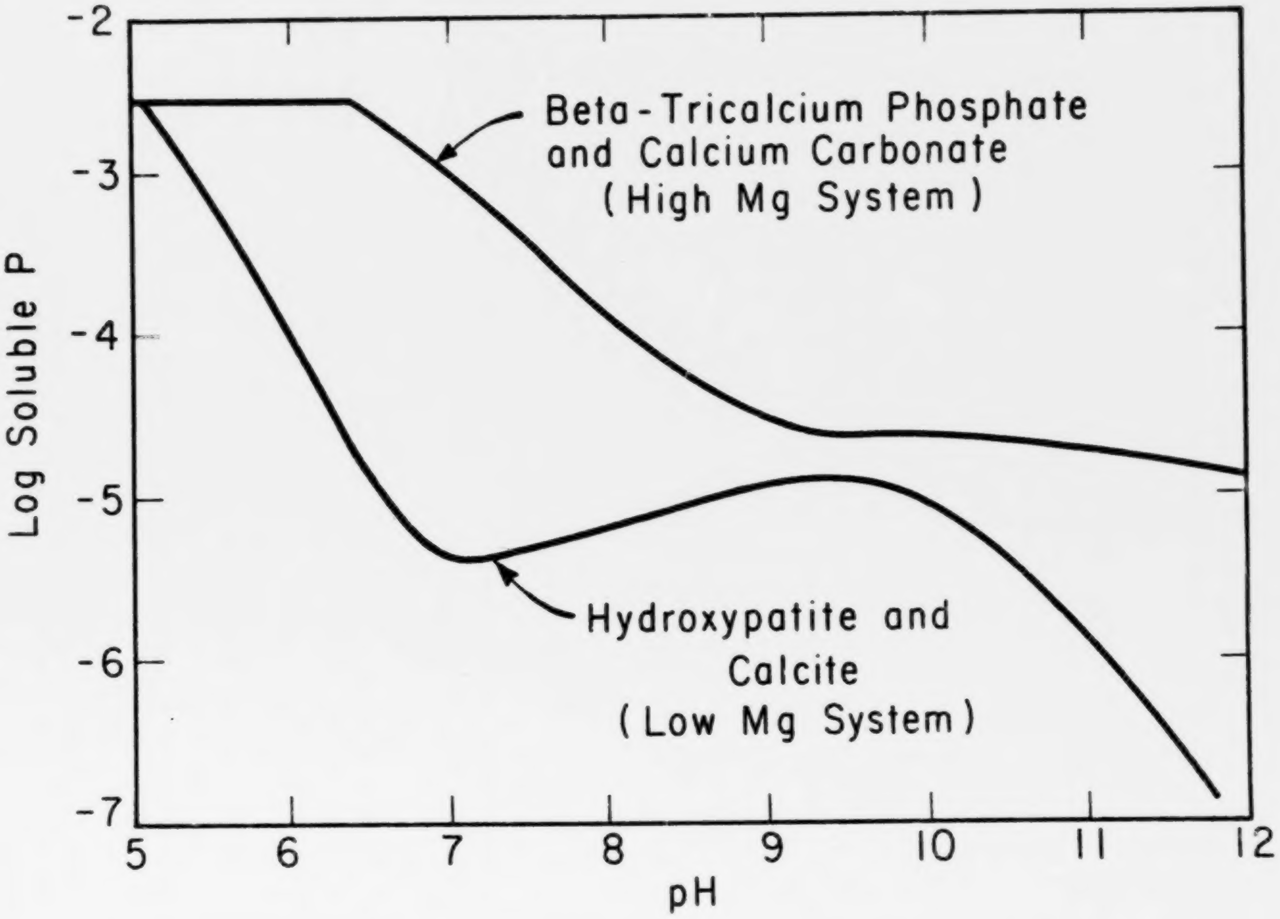

Figure 3. Predicted Soluble Phosphate at Equilibrium for Systems with High and Low Magnesium Concentrations (PO_{4t}= 0.003 M, CO_{3t}= 0.05 M, Ca_t= 0.07 M, T= 25°C) (after Ferguson and McCarty).

hydroxyapatite from solutions containing solid calcium phosphate and calcium and phosphate ions. In both cases the hydroxyapatite crystals formed were very small and did not grow. Reflux of the precipitates did not result in increased perfection of the crystals and growth was negligible after 90 hours. Leckie and Stumm (1970) reported that formation of hydroxyapatite occurs in two steps:

1) the formation of nuclei; and
2) the two dimensional growth of crystals on the surface.

The second step is time-consuming, but can be reduced greatly if apatite nuclei are present. Calcite, silica and undefined solids in domestic wastewater also can act in this capacity (33).

The slow growth of crystals experienced by Corsaro, _et al_. and Leckie and Stumm can explain the higher soluble residuals found by Ferguson and McCarty in short-term tests. Neither Corsaro or Ferguson and McCarty had any apatite nuclei available and the precipitates formed were subject to the slow two dimensional growth described by Leckie and Stumm.

Ferguson, _et al_. (37) investigated the precipitation of calcium-phosphorus compounds under conditions of pH and concentration similar to those found in domestic wastewaters. Figure 4 depicts the results of one series of tests, and shows the time lag, or induction period, described by Leckie and Stumm at pH 7.6. In another experiment they added the precipitate formed in a previous test to observe the effect of "seed" being present. The presence of the previously formed solid caused immediate precipitation with approximately first order kinetics and there was no induction period.

Fluoroapatite: There are still other factors that can affect the rate of precipitation. Leckie and Stumm (33) reported that fluoride, even in trace concentrations (less than 1 mg/l), enhances the rate of precipitation, presumably because fluoroapatite, which is less soluble than hydroxyapatite, is being formed. More significantly, because F^- can substitute for OH^- in the apatite structure, adding F^- has an effect similar to raising the pH. Within the pH range normally encountered in activated sludge systems, 7.0 to 8.5, the solubility is an order of magnitude lower than hydroxyapatite.

Fluoroapatite could be a significant factor in phosphorus removal due to the natural presence of fluoride, as well as to its addition to many water supplies for dental health in concentrations that can enhance precipitation (1 mg/l).

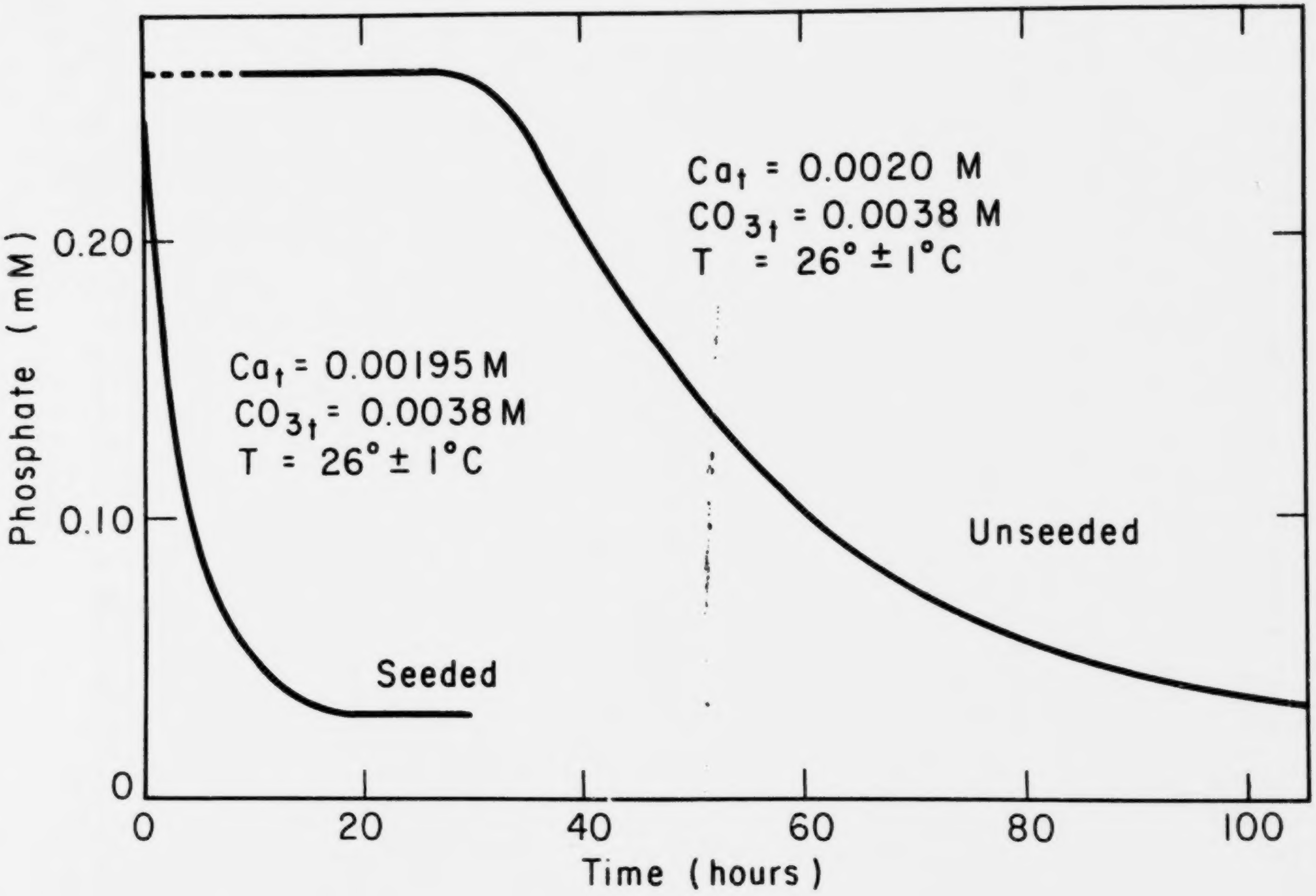

Figure 4. Effect of Time on Phosphate Removal at pH 7.6 with Seeded and Unseeded Solutions (after Ferguson, et al.).

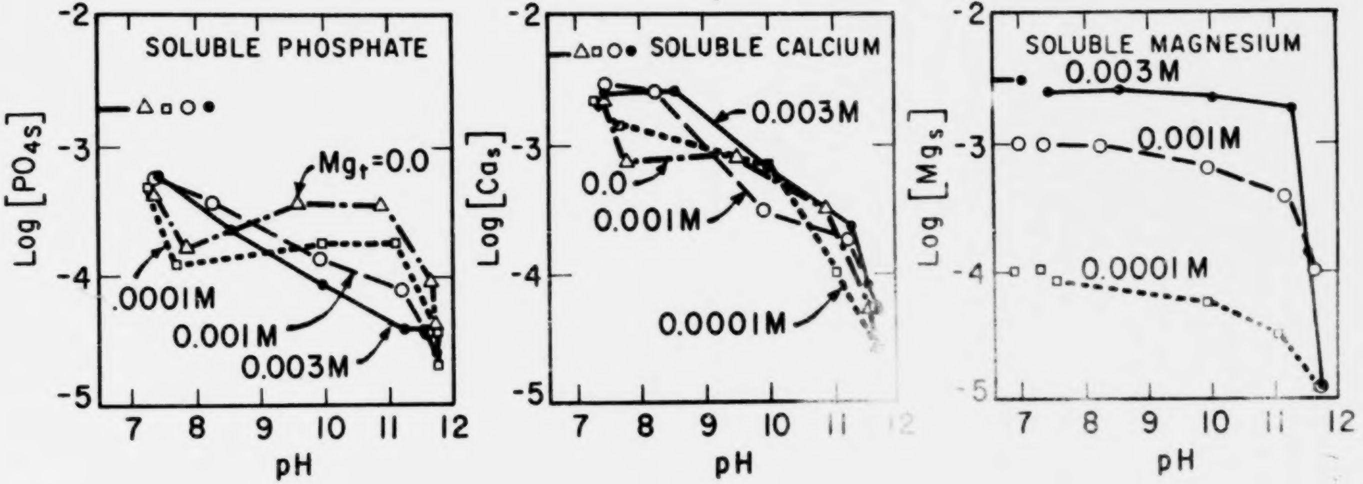

Figure 5. Effect of Magnesium Concentration and pH on Soluble Phosphate, Calcium and Magnesium (CO_{3t}= 0.03 M, PO_{4t}= 0.002 M, Ca_t= 0.005 M, T= 27°C, time at 24 hrs.) (after Ferguson and McCarty).

Beta-Tricalcium Phosphate

In their theoretical study Ferguson and McCarty (35) predicted that beta-tricalcium phosphate would precipitate in solutions containing magnesium. This compound has received little attention in past studies of suspected solid calcium-phosphorus phases in wastewaters. Beta-tricalcium phosphate ($B\text{-}Ca_3[PO_4]_2$), has been found to precipitate, but the presence of other ions is needed to stabilize its formation (38). Magnesium stabilizes the formation of beta-tricalcium phosphate, and is often included about 6 to 8 percent. Other impurities also stabilize it, such as manganese and ferrous iron. From their theoretical model, Ferguson and McCarty (35) predicted that the calcium-phosphorus precipitate could be beta-tricalcium phosphate in preference to hydroxyapatite for the Ca:Mg ratio found in most fresh and wastewaters (1.5 to 6) (see Figure 2).

In their experimental tests magnesium did alter the phosphorus residual below pH 9, and the minimum phosphorus solubility reported for the hydroxyapatite system between pH 7 and 8 did not exist (see Figure 5). With no magnesium present this minimum was near 10^{-4}M, but with magnesium ($[Ca^{++}] = 5 \times 10^{-3}$M initial conditions) was near 5×10^{-3}M. The lowest values for Ca:P ratios in the samples without magnesium approached 1.67, the value for hydroxyapatite; while in the magnesium samples, the Ca:P ratio approached 1.5, the value for beta-tricalcium phosphate.

X-ray and Infrared Absorption Measurements

Yu and Mark (39) used infrared absorption to analyze precipitates obtained from solutions of $CaCl_2$ and $(NH_4)_2HPO_4$ in pure solution, and in the presence of a surface active agent (p-nitrophenol) and blue-green algae. The results are shown in Figure 6. The precipitates from several tests, including some using samples of recirculating sludge from anaerobic digesters, also were analyzed by Ferguson and McCarty (35) using infrared absorption and the spectra obtained are reproduced in Figure 7. Table 2 contains the characteristics of these samples. The 965 cm^{-1} band is of interest. Studies have shown that this peak in the spectra of hydroxyapatite can be used to measure the degree of crystallinity (40).

Ferguson and McCarty's samples 1,2 and 3 (without magnesium) in Figure 7 show the same 965 cm^{-1} peak as that of Yu and Mark's sample in pure solution in Figure 6. The samples of Yu and Mark with p-nitrophenol and blue-green algae present show the shoulder only indicating no ordered

Table 2

Composition of Solutions Used in Infrared Analyses

After Yu and Mark (see Figure 6)

1. Equal Parts of 0.75 M $CaCl_2$ and 0.25 M $(NH_4)_2$ HPO_4

2. 500 ml 0.75 M $CaCl_2$, 500 ml 0.25 M $(NH_4)_2$ HPO_4, and 700 ml 0.02 M p-nitrophenol

3. 500 ml 0.75 M $CaCl_2$, 500 ml 0.25 M $(NH_4)_2$ HPO_4, and 700 ml of an algae solution

After Ferguson and McCarty (see Figure 7)

							Precipitated			
Sample	Ca	P	Mg	CO_3	pH	COD (mg/l)	CO_3	Ca	P	Mg
1. pure sys.	5.0	2.0	0	30	7.91	--	1.65	3.81	2.03	--
2. pure sys.	5.0	2.0	0	30	10.06	--	2.69	4.48	1.72	--
3. pure sys.	5.0	2.0	0	30	11.46	--	2.44	4.66	1.95	--
4. pure sys.	5.0	2.0	2.0	30	8.11	--	1.35	3.52	2.57	.29
5. pure sys.	5.0	2.0	2.0	30	10.06	--	2.74	4.55	2.95	.88
6. pure sys.	5.0	2.0	2.0	30	11.45	--	2.96	4.95	2.45	1.92
7. M.P.S.L.*	6.0	2.2	2.7	28	7.35	214	0.1	2.21	1.29	.23
8. M.P.S.L.	6.0	2.2	2.7	28	9.94	99	3.65	5.20	2.06	.92
9. M.P.S.L.	6.0	2.2	2.7	28	11.25	186	4.25	5.41	2.08	2.47

* Menlo Park Supernatant Liquor; concentrations in mMoles per Liter.

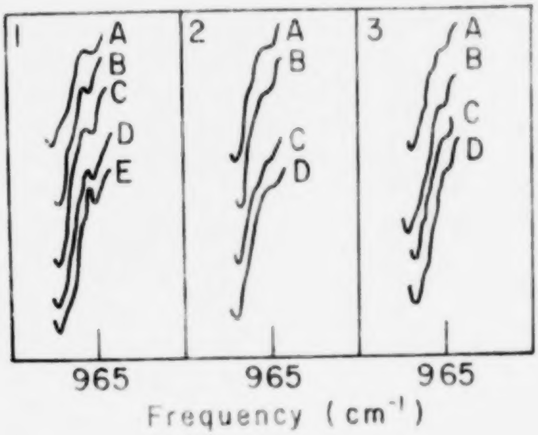

Figure 6. Infrared Spectra of Hydroxyapatite Precipitates. Left, formed in pure H_2O: A, 18 hrs.; B, 3 days; C, 4 days; D, 7 days; E, 9 days. Center, effect of p-nitrophenol: A, 1 day; B, 3 days; C, 6 days; D, 9 days. Right, effect of blue-green algae: A, 1 day; B, 5 days; C, 10 days; D, 20 days (after Yu and Mark).

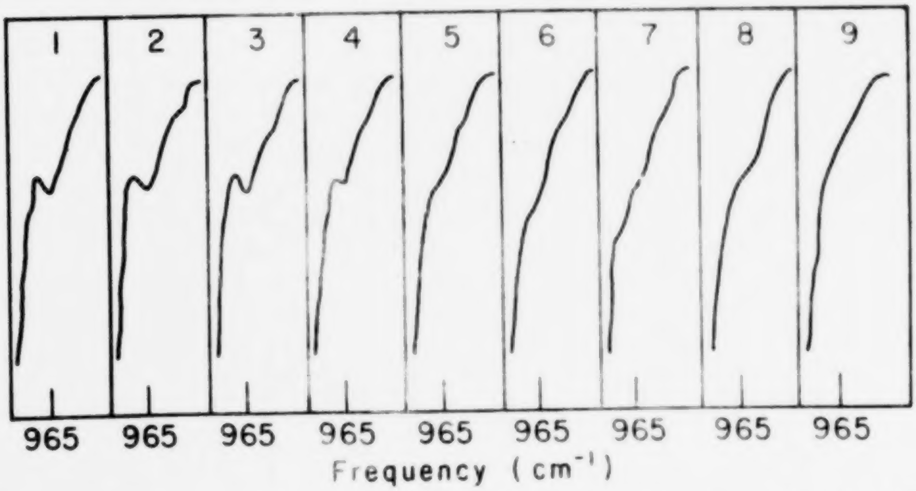

Figure 7. Infrared Spectra for Samples Precipitated for 24 hrs. See Table 2 for Composition (after Ferguson and McCarty).

crystals, but amorphous hydroxyapatite. Samples 4,5 and 6 with magnesium show: a small peak, a shoulder only, and little, if any, shoulder. These samples have a Ca:Mg ratio of 2.5. From these results it would appear that Ferguson and McCarty's sample 4,5 and 6 did not have well ordered crystaline apatite but an amorphous apatite. Samples 7 and 8 (from anaerobic sludge) show a small shoulder indicating an amorphous form. Sample 8 (Ca:Mg ratio of 2.2) and sample 9 (Ca:Mg of 2.2) show no shoulder and could indicate no apatite present.

Martens and Harris (41) studied the effect of magnesium on the direct precipitation of apatites in marine environments. They reported that magnesium inhibits the formation of apatite, and that the critical Ca:Mg ratio where this occurs is between 4.5 and 5.2. Above this value they obtained X-ray diffraction patterns with strong apatite peaks, and below they obtained no indications of apatite. The pH range was maintained between 7.5 and 8.0. They did not report the Ca:P ratios of the precipitate above the critical Ca:Mg ratio, but reported a ratio of 1.35 below.

Ferguson and McCarty defined a magnesium influenced system when the Ca:Mg ratio is 5 or less, and Martens and Harris defined the ratio between 4.5 and 5.2 or less. Both investigators obtained Ca:P ratios that indicate a solid form other than apatite. The theoretical and actual increase in residual phosphorus solubility formed by Ferguson and McCarty would seem to point to beta-tricalcium phosphate.

Effect of Operational Characteristics on Phosphorus Removal

Several prototype and pilot plant as well as laboratory studies have been conducted to investigate the abnormal phosphorus removal capabilities and sludge phosphorus content found in several activated sludge plants. Some of these studies have involved evaluation of the operational characteristics of plants enjoying high removals of phosphorus and subsequent attempts to duplicate these characteristics of plants in other plants having normal phosphorus removal (42-49). Other studies were pilot plants with associated specific laboratory experiments to verify the significance of these operational characteristics (3,34). The major implicated characteristics are:

1) dissolved oxygen (DO) levels along and at the effluent end of the aeration tanks;
2) the organic loading, pounds of applied BOD per pound of solids under aeration; and
3) calcium, pH and other ionic constituents.

Prior to evaluating the results of these field studies, a discussion of the parameters used to define the removal of phosphorus from wastewaters would allow a more meaningful interpretation of the results of these studies.

Evaluation of Phosphorus Removal Measurements

The reporting of influent phosphorus as soluble ortho-phosphate is misleading. The quantity of condensed and organically bound forms of phosphorus may equal the quantity of orthophosphate present in the influent. The biomass can hydrolyze the condensed forms rapidly as well as release the bound forms of phosphorus for metabolic purposes during the treatment cycle. As a result the influent phosphorus concentrations reported as ortho-phosphate can be significantly less than that actually available to the biomass. Erroneous percent phosphorus removal values also can occur from reporting the effluent phosphorus concentrations as soluble ortho-phosphate. The reporting of all forms of phosphorus (total P) present in the waste stream and all soluble forms (total filtrate P) in the effluent of the plant would better describe the quantity of phosphorus removed from the wastewater.

The practice of reporting phosphorus removal on a percentage basis is questionable. A treatment plant experiencing 80% P removal having an influent containing 10 mg/l P cannot be compared to other plants with 80% P removal having influents cycling from 5 to 15 mg/l P where the phosphorus removed from the waste streams would range from 4 to 12 mg/l P.

Moreover, the percent phosphorus removed from a waste input does not describe the phosphorus removal capability of the sludge beyond that particular treatment plant and wastewater characteristics. Assuming only biological uptake of phosphorus, part of the removal of phosphorus would be to satisfy the metabolic requirements of the biomass. This removal might be directly related to the removal of other nutrients, i.e. organic carbon expressed as BOD or COD. Some investigators (15,3) have related the uptake of phosphorus to the BOD or COD removed to define the phosphorus removal capabilities of sludges on a common basis (pounds phosphorus removed per 100 pounds of BOD or COD removed).

The last area of possible confusion is the lack of reporting or neglecting sludge phosphorus content. Without a knowledge of initial and final sludge phosphorus content the results of most studies, and in particular batch-type systems, have little meaning.

Effects of Dissolved Oxygen

The operational characteristic receiving the most attention in both field and laboratory studies has been the level of dissolved oxygen (DO). Without particular reference to the biological uptake of phosphorus,this is indeed an important characteristic of any aerobic biological system. The reports of several field investigations on activated sludge plants with abnormal phosphorus removal capabilities have assigned major significance to the maintenance of DO levels in the aeration tank and effluent much in excess of those previously reported for oxygen to be non-limiting.

Vacker, et al. (4) attributed this increased phosphorus removal enjoyed by the Rilling plant above the East and West plants at San Antonio to several factors, but noted that DO levels were a major difference in the operating characteristics between the plants. These three plug-flow activated sludge plants are at a common site and receive the same wastewater for treatment. The Rilling plant exhibited DO levels of 2 mg/l or greater during the last half of the aeration tank length, and usually had a DO in the aeration tank effluent near 5 mg/l. The East plant seldom exceeded DO levels of 3.0 mg/l in the effluent and usually dropped to a level of 1.0 to 2.0 mg/l during peak loading periods. The West plant was similar, but usually fell to lower levels than in the East plant. The East and West plants contained an average of 3.7 to 6.0 mg/l $O\text{-}PO_4$ as P opposed to 1.9 mg/l $O\text{-}PO_4$ as P in the Rilling plant effluent against a common influent containing 9.1 mg/l $O\text{-}PO_4$ as P. In the report of average daily operation data for 1965 the phosphorus removed at Rilling averaged 7.3 mg/l $O\text{-}PO_4$ as P, East averaged 5.5 mg/l $O\text{-}PO_4$ as P, and West averaged 3.3 mg/l $O\text{-}PO_4$ as P. The calculated percent sludge phosphorus reported for the plants was 6.2, 3.7, and 2.8 percent respectively.

Witherow (5) reported the results of incorporating the operating characteristics of the Rilling plant at the West plant in an attempt to induce high phosphorus removals. High phosphorus removals were obtained, but required the removal of two tanks with inefficient air diffusers to obtain DO levels similar to Rilling. During the test the operational changes affected the aeration solids level (increase from 900 to 1800 mg/l MLSS), control of organic and hydraulic loading, solid-liquid separation (2 to 4 mg/l polyelectrolyte added to prevent bulking), and the DO level in the aeration tanks.

While these changes closely duplicated the characteristics of the Rilling plant, similar uptake of phosphorus at the West plant had been noted by Vacker, et al. (4) to occur with changes in the DO level only. They reported the results of increased aeration efficiency during a periodic cleaning of the diffusers at the West plant. The cleaning of the diffusers was accomplished in a manner that permitted the approximation of tapered aeration and reversed tapered aeration, as well as one tank with all diffusers cleaned. Loadings and solids level were the same as normally present, solids varied between 1510 mg/l at low flow and 810 mg/l at peak flow. Normal removal occurred in the one tank with no diffusers cleaned, but increased removal occurred in the three other partially and fully cleaned tanks with effluents containing 1.5 mg/l O-PO_4 as P or less. Comparing these two tests would seem to indicate the significance of DO levels as the primary characteristic influencing phosphorus removal capability of the West plant sludge. It also should be noted that the pattern of air distribution was not highly significant, only the maintenance of DO levels exceeding 2 mg/l along most of the aeration tank length.

A study was conducted at the Baltimore Back River Sewage Works (42) to evaluate the high phosphorus removal obtained in the activated sludge plant under operating conditions similar to the San Antonio Rilling plant. The effect of DO levels again was noted. In a test, three jugs containing 15 liters of mixed liquor from the influent end of the aeration tank were aerated at three levels of air flow, 5, 10, and 15 liters per minute. Simultaneously, a slug of waste was monitored through the aeration tank on the basis of the five hour detention time determined by dye tracer studies. The jugs and the slug of waste exhibited the same phosphorus removal, approximately 24 mg/l O-PO_4 as P, and had residual O-PO_4 concentrations less than 0.1 mg/l P. Again the rapid uptake of phosphorus appeared to occur when the DO levels began to increase above the initial values, although not as clearly as at San Antonio.

The results of these field evaluations of activated sludge plants enjoying high phosphorus removals implicate DO levels as a significant operational characteristic. However, attempts to verify this in other field and laboratory tests have not met with success.

Menar and Jenkins (34) conducted laboratory and pilot plant studies of operating conditions that enhance phosphorus removal. Of particular interest in their study was the effect of DO levels. They concluded that the DO level *per se* did not enhance the removal of phosphorus, but is fortuitously correlated to conditions indicating a rise in pH, i.e. the stripping of CO_2, and that enhanced phosphorus removal occurs as the result of chemical precipitation.

A pilot plant was operated that incorporated the operational characteristics reported as significant in the San Antonio studies. Influent to the San Ramon, California, Valley Community Services District was used in the study. This waste had characteristics similar to the San Antonio waste with the exception of magnesium, 44 mg/l versus 17 mg/l and alkalinity, 405 mg/l versus 225 mg/l as $CaCO_3$. (These higher values at San Ramon may have exerted an effect and will be discussed later.) The physical layout of the pilot plant closely resembled the two-pass aeration basin of the Rilling plant.

During the six week test the DO level was maintained consistently above 1.5 mg/l. The phosphorus removed during this time was a function of the amount of carbon removed as defined by Sawyer (1), Helmers, et al. (12,13,14) and Jenkins and Menar (3), i.e., approximately 0.9 pounds phosphorus per 100 pounds of COD removed. The maximum sludge phosphorus content was 2.9 percent and averaged 2.3 percent (based on volatile solids, VSS), which agrees with the above that phosphorus removal is a function of carbon removal.

The operating characteristics then were changed to include a preaeration basin. In addition to a higher DO, the pH was raised allowing the condensed phosphates to hydrolyze and release the bound calcium. The preaeration time was 4.3 hours and normal aeration time was 4.4 hours. During preaeration the pH rose from 7.7 to 8.3 and maintained this level through normal aeration. The DO level of the mixed liquor in the preaeration basin increased to 5.4 mg/l, and subsequent normal aeration increased this to 6.2 mg/l at the effluent end.

These conditions produced enhanced phosphorus removal, approximately double that expected from normal biological incorporation. The sludge phosphorus content reflected this increase and averaged 4.5 percent. Calcium also was removed from the wastewater, 8.0 mg/l. Menar and Jenkins concluded that chemical precipitation was the cause of the enhanced phosphorus removal. Attempts to define the characteristics by X-ray diffraction were not successful.

Other studies were conducted with an effluent solids level (from 2000 to 5000 mg/l MLSS). These subsequent tests were conducted under conditions typical of the extended aeration process. The mean residence times of the sludges exceeded 12 days and substrate removal rates were 0.2 to 0.25 pounds COD per pound of VSS. With preaeration and its effect on pH, two to three times the expected phosphorus removal occurred with sludge phosphorus content reflecting

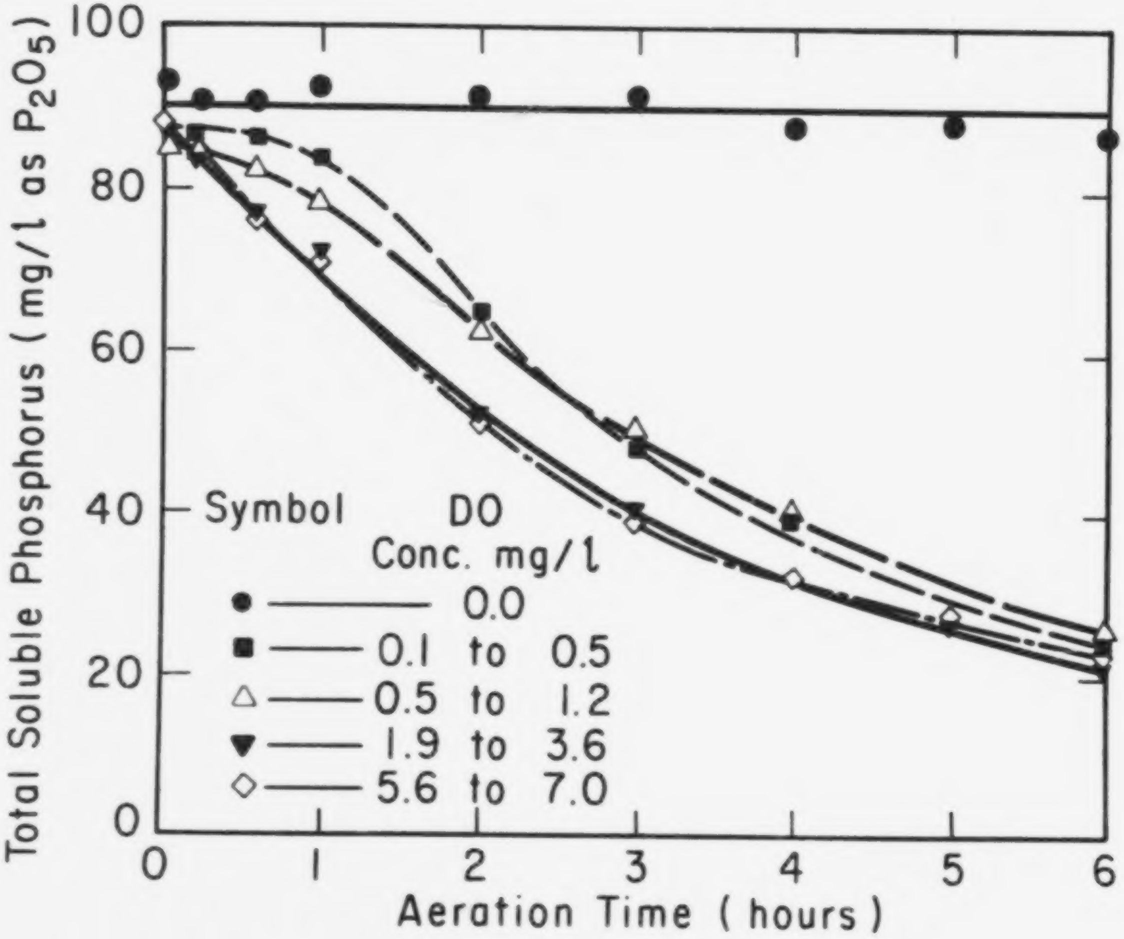

Figure 8. Soluble Phosphate Uptake as a Function of Dissolved Oxygen Concentration (after Hall and Englebrecht).

the increased removal. Calcium also was removed from the wastewater and the sludge calcium content increased in relation to the increased phosphorus removal. An average of 6.8 percent phosphorus and 10 percent calcium was reported for the sludge.

Hall and Englebrecht (20) used fill and draw laboratory units to observe the effect of DO on the uptake of phosphorus. They used mixtures of nitrogen and oxygen to provide varying DO levels, but with the same turbulence in each unit. Their results are presented in Figure 8. With DO levels below 2 mg/l there is a lag period; however, after six hours there is little difference in the amount of phosphorus removed.

A recent investigation of the Baltimore plant by Milbury, et al. (50) evaluated the effects of DO levels in the aeration tanks. They reported that the DO levels in the aeration tanks did exert some effect on phosphorus removal, but at lower levels than previously reported. They concluded that a DO of 1.0 mg/l at the two-thirds point along the aeration tank length and an effluent with 2.0 mg/l DO, no impairment of phosphorus uptake would occur. The rapid uptake of phosphorus did not always occur concurrently with an increase in DO or an abrupt increase in pH. The pH range varied from 6.8 to 7.3 and always increased through the aeration tanks, even when little phosphorus removal occurred. This high phosphorus removal at approximate neutral pH conditions indicated to these investigators either excess biological uptake or a solid species of phosphorus not as pH dependent as those postulated by Jenkins and Menar.

Furthermore, a batch test was conducted to observe the effects of DO level under laboratory conditions. They reported no impairment of phosphorus uptake at DO levels above 0.5 mg/l.

Organic Loading and Aeration Solids

Vacker, et al. (4) implicated the organic loading as exerting an effect on the phosphorus removal capability of activated sludge as a result of their study at the San Antonio plants. Significance was attached to a loading rate around 0.5 pounds of applied BOD per pound of MLSS; however, this was apparently as much towards maintaining a balance between sludge wasting, MLSS, and rates of sludge return to keep aerobic digestion of solids to a minimum, thus preventing phosphorus return to the mixed liquor.

Witherow (5) obtained the organic loading rates presented from the rates existing in the high phosphorus removal plants when abnormal removals were occurring. The loading rates from normal phosphorus removal plants investigated were generally lower. There was some deviation from the optimum range, 0.26 to 0.35 pound BOD per pound MLSS, in the high removal plants when they were experiencing low removals.

Hall and Englebrecht (20) used fill and draw laboratory units to investigate the effects of organic loading and MLSS on phosphorus removal. Their tests spanned a range of loading rates from 0.33 to 4.0 and MLSS from 750 to 8000 mg/l. These variations were obtained by maintaining a constant substrate concentration and varying MLSS during one series and by maintaining a constant MLSS and varying the substrate concentration in another series. During both series they maintained a constant COD:P ratio of approximately 75:1 so that the rate of phosphorus uptake was not affected. No effect from either the organic loading or MLSS was noted.

Hall and Englebrecht maintained a stock culture that was fed a substrate composed of glucose and yeast extract for carbon sources and the normal mineral content found in laboratory media. Chemical precipitation was precluded as a significant phosphorus removal mechanism based on substrate content. From the data available it was possible to compute a yield coefficient for the stock culture, 0.53, and this is within the range expected for such a medium. Using this yield coefficient and the data on phosphorus and COD removal, it was possible to compute a cellular phosphorus content that would be close to the actual value reported. This was done for both series and was found to range between 2.35 to 2.5 percent for all tests. The ratio of phosphorus removed to COD removed was 1.2 pounds P per 100 pounds COD removed.

These tests did not indicate any effects from varying organic loading or MLSS over a range that would represent most conditions found in activated sludge plants. The sludge phosphorus contents obtained were within the normal range for activated sludge.

Jenkins and Menar (3) investigated organic loadings over a large range, 0.2 to 13.8 pounds BOD per pound VSS, in a pilot plant study. The sludge phosphorus content did not vary significantly over this rather large range and had a weighted average of 2.62 percent for all loading rates. From 0.2 to 3.0 pounds BOD per pound VSS, the ratio of phosphorus removed to COD removed was 0.86. Above these loadings the ratio increased to 1.7 at the highest loadings.

These investigators concluded that organic loading in the range found in most activated sludge plants, 0.2 to 3.0, did not exert an effect on phosphorus removal.

Witherow (5) reported the results of field evaluations of several activated sludge plants. There was an optimum solids level indicated, between 2000 and 4000 mg/l MLSS, but the results were based on changes in MLSS only and were not related to the changes in organic loading resulting from these changes in aeration solids. The decline in removal at high MLSS levels probably reflected the low organic loading present which would induce a loss in VSS with a release of phosphorus. This was noted by Vacker, et al. (4) at San Antonio and by Jenkins and Menar (3) to occur at loading levels below 0.2 pounds BOD per pound MLSS.

Calcium, pH and Other Ionic Constituents

Vacker, et al. (4) assigned major significance to a biological removal of phosphorus at San Antonio, but did report that a removal of hardness, i.e. calcium, occurred through the aeration tank. This removal was small, and it was not assigned major significance.

It is interesting to note the stoichiometric requirements in the concentration realm of interest in activated sludge systems. The quantity of phosphorus to be removed is usually between 6 to 12 mg/l P (2×10^{-4}M to 4×10^{-4}M). Considering the two main suspected solid forms and their Ca:P ratios: hydroxyapatite, 1.67, and beta-tricalcium phosphate, 1.5; the required amount of calcium is between 12 and 27 mg/l Ca (3×10^{-4}M and 7×10^{-4}M). Vacker, et al. reported the calcium content of San Antonio fresh water varied between 59 and 90 mg/l (1.5×10^{-3}M and 2.25×10^{-3}M). These reported values do not account for the insoluble or bound forms of calcium that are released to solution in the treatment plant. The small amount of calcium required for significant removal of phosphorus could be within the normal variation encountered and be masked by this variation.

Menar and Jenkins (34) reported chemical precipitation in their pilot plant study using San Ramon wastewater, where the Rilling plant exhibited abnormal phosphorus uptake. The enhanced phosphorus removal, up to three times that required for the carbon removed, was directly tied to the removal of a precipitating cation, calcium. But the pH level required to induce the chemical precipitation was far in excess of the values reported for San Antonio and other plants with abnormal phosphorus removal capability. The pH levels attained at San Ramon were greater than 8 while San Antonio achieved abnormal phosphorus removals at pH 7.6 and Baltimore (50) at a pH near 7.0.

Recent reports by Ferguson and McCarty (35), Ferguson, et al. (37) and Leckie and Stumm (33) have provided possible explanations for the dilemma occurring with the wide pH range encountered in reports of enhanced phosphorus removal. Ferguson and McCarty reported that two solid forms would precipitate from calcium-phosphorus systems; apatite and beta-tricalcium phosphate. The condition influencing the form precipitated was a function of other ions, notably magnesium. If magnesium was present in sufficient concentration so that the calcium : magnesium ratio was less than five, the precipitation of beta-tricalcium phosphate would be favored. Should the conditions for the precipitation of beta-tricalcium phosphate occur, the resulting soluble fractions of calcium-phosphorus systems that can exist in the pH range from 7.0 to 9.0 provide possible explanations for the observations at several activated sludge plants.

As pointed out by Ferguson and McCarty the formation of beta-tricalcium phosphate changes the residual soluble phosphorus, particularly between pH 7.0 and 8.0 where the residual soluble increases significantly over that found where apatite is the solid form. This effect could have caused the results obtained by Menar and Jenkins (34) in their pilot plant study at San Ramon, California. In duplicating the characteristics and conditions found at the San Antonio Rilling plant they did not attach any significance to two differences in the composition of the wastewaters: the magnesium content of the San Ramon wastewater was close to three times that of San Antonio wastewater and the alkalinity was almost double. Should this high magnesium content have inhibited apatite and favored the formation of beta-tricalcium phosphate, this would have caused higher soluble phosphorus residuals than predicted for an apatite system and could have precluded any significant precipitation at the calcium and phosphorus concentrations present. To obtain a significant calcium-phosphorus precipitation the pH had to be increased to levels above 8.0. While they did cause precipitation to occur, by increasing the pH through CO_2 stripping via increased aeration rates (thereby raising the dissolved oxygen) and raising the sludge phosphorus content to a high of 6.8 percent, the soluble phosphorus residual was still quite high, almost 0.3×10^{-4}M (~10mg/l P). This high residual could have been influenced by not only the effect of magnesium, but also by the competitive precipitation of calcite (35) which was significantly greater at San Ramon at the pH encountered.

Secondly, the implication of fluoride in the enhanced precipitation of apatites as pointed out by Leckie and Stumm can lower the pH range for significant removal by precipitation. The concentration found in municipal water supplies adding fluoride for dental health as well as that occurring naturally in the range of significance (~1 mg/l F^-) reported by Leckie and Stumm. Several cities reported having abnormal phosphorus removal containing fluoride at this concentration according to the Geological Survey Water-Supply Paper 1812 (1962). Table 3 is a listing of several activated sludge plants reporting high phosphorus removal. These include Baltimore, 1.0 mg F^-/l and Amarillo, 2.0 mg F^-/l. The presence of fluoride in the Baltimore plant could account for the high phosphorus removal at the low pH range, 6.8 to 7.2, reported by Milbury, et al. (50).

Table 3

Chemical Analyses of Fresh Water Supplies of Some Municipalities Reporting High Sludge Phosphorus Content
Values are in Milligrams per Liter
(Taken from Geological Survey Water-Supply Paper 1812, [62]).

	Ca	Mg	HCO_3	F
Amarillo	38	30	275	1.9
Baltimore	18	3	45	1.0
Fort Worth	45	8	146	.3
Los Angeles	42	12	135	.6
San Antonio	62	16	240	.3

Literature Evaluation

In the previous sections the two viewpoints postulated to identify the cause of abnormal uptake of phosphorus by some activated sludge plants beyond the amounts described in nutrient requirement studies (1,12,13,14) have been reviewed. There is still insufficient evidence to positively determine the primary mechanism(s) responsible; a biological uptake termed "luxury uptake", or direct chemical precipitation and subsequent sorption or enmeshment in the activated sludge mass.

The result of field and laboratory studies can partially validate both mechanisms and the following preliminary conclusions are postulated.

1) The lower limit of sludge phosphorus content is approximately one percent for good operation of activated sludge systems based on carbon removal. The cellular phosphorus content that normally can be expected due to biological uptake when excess phosphorus is available to a biomass is between two and three percent and appears related to improved solid-liquid separation. The upper limit of biological assimilation of phosphorus has not been defined, though, and has been postulated to exceed this range by as much as twice in some activated sludge plants.
2) A possible means of determining the extent of biological phosphorus assimilation is the alkaline phosphatase bioassay.
3) The two main suspected calcium phosphate precipitates are apatites and beta-tricalcium phosphate.
4) The possibility of a chemical removal mechanism exerting a significant effect on the quantity of phosphorus removal in activated sludge plants cannot be refuted, but the wide pH range reported presents a more complex problem in describing the existence and forms of this removal mechanism. The nature of the precipitate formed, a colloid, plus the small concentrations of ions involved pose problems in definitively describing its existence by physical means. The inability to identify the presence of precipitates in sludges by X-ray or infrared techniques has not aided the proponents of this removal mechanism, even though the presence of contaminants, such as found in activated sludge, has been shown to obscure the presence of precipitates.
5) The complexities of extraneous cations purported to remove phosphorus by chemical precipitation,as well as other uncontrollable conditions (such as pH, variable ionic concentrations in cyclic flow, etc.) preclude the use of field experiments to quantitatively evaluate metabolic incorporation of phosphorus and the chemical precipitation of phosphorus with calcium.

Objective and Scope

The objectives of this research are to evaluate the range of metabolic phosphorus removal, and to investigate the significance of calcium removal of phosphorus at ionic concentrations and pH generally encountered in domestic wastewater treatment plants. To accomplish this purpose it will be necessary to:

1) define the range of cellular phosphorus content; and
2) define the chemical conditions that induce phosphorus removal by precipitation with calcium.

The complexities of contributing factors that can exert a significant effect have been delineated previously. Control of all these factors is not possible in field tests; therefore, only laboratory experiments were conducted in order to permit their inclusion and evaluation in this investigation.

The laboratory experiments were designed to provide a means of differentiation and evaluation of the magnitude of phosphorus removed by each mechanism. The scope of the study included the following components.

1) Investigation of the range of metabolic incorporation of phosphorus in bench scale completely mixed continuous flow activated sludge systems using a synthetic substrate representative of actual wastewater under conditions precluding significant chemical removal.
2) Concurrent with determining the magnitude of metabolic incorporation of phosphorus as described above, evaluating the alkaline phosphatase bioassay technique as a means of defining the range of biological phosphorus uptake and the saturation value of cellular phosphorus content in a laboratory activated sludge system.
3) Simultaneous operation of bench scale completely mixed continuou flow activated sludge systems under pH, ionic concentrations and chemical conditions that both favor and preclude significant chemical removal of phosphorus with calcium as the precipitating cation. The effect of high and low Ca:Mg ratios, the presence of 1 mg/l F^- in the influent, and the addition of nucleation sites were studied.

SECTION IV

EXPERIMENTAL PROCEDURES

The first part of this section describes the apparatus and procedures employed during the experimental studies. The analytical techniques are described in the second section.

Experimental Studies

Both batch and completely mixed continuous flow activated sludge units were utilized for the biological studies. Differentiation between metabolic incorporation and chemical precipitation of phosphorus within the sludge was accomplished using synthetic substrates with defined calcium and phosphorus concentrations. Appropriate pH ranges were maintained by using an automatic pH control system, so that significant calcium precipitation of phosphorus did, or did not, occur. The concentration of calcium and phosphorus and the pH ranges that precluded or favored calcium precipitation of phosphorus were determined by batch chemical precipitation tests.

Batch Units for Biological Studies

A plexiglass unit was constructed providing five separate compartments. Each compartment was 3 x 5 x 15 inches with a sampling port one inch from the bottom. Aeration was provided by a one inch diffuser stone permanently mounted one inch from the bottom opposite the sampling port. The initial test volume was 2.5 liters.

The desired concentration of substrate for a particular experiment was added to a unit partially filled with distilled water. Subsequently a concentrated inoculum of activated sludge was added, and the unit was brought to the final test volume with distilled water. High aeration rates were used to maintain the solids in suspension and to maintain saturated dissolved oxygen conditions.

Completely Mixed Continuous Flow Units for Biological and Chemical Studies

A sketch of the plexiglass completely mixed continuous flow unit is shown in Figure 9. The units provided aeration and settling sections

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separated by a moveable baffle with an overall test volume of 8 liters. Solid-liquid separation was accomplished by gravity settling and subsequent return of the solids to the aeration section by hydraulic action.

A diffuser stone was suspended from a moveable platform over the aeration section. This was used primarily to provide oxygen, although the positioning of the stone also was used in conjunction with the moveable baffle to provide the necessary hydraulic action to return the solids from the settling section. No difficulties were encountered in providing saturated dissolved oxygen conditions, maintaining suspension of solids, and returning settled solids during normal operation. During some tests sludge bulking presented problems in returning solids to the aeration section. These occurrences are reported in experimental results.

A variable speed tubing pump (Cole-Palmer Masterflex) was used to deliver the substrate to the units. Initially an 18 liter plastic feed tank was used to provide 2 days of feed; however, during the first series of biological tests contamination was noted in several feed tanks. This was corrected by changing to a twice daily feed schedule using 4 liter aspirator bottles.

Prior to sampling or wasting of sludge, the baffle was removed and the contents of the aeration and settling sections allowed to mix. The variability in the quantity of solids on the settling section necessitated this action to maintain a common reference for solids measurements in the units.

Inoculum

The source of inoculum for the biological studies was the Govalle Wastewater Treatment Plant, Austin, Texas. The sludge was screened to remove large discrete particles, elutriated with distilled water to remove grit and nutrients present in the occluded wastewater, then concentrated by gravity settling.

When low phosphorus content sludge was required, the sludge was placed under anaerobic conditions for 24 hours subsequent to the above pretreatment. Sludge phosphorus content was approximately 2 percent. After this period 750 mg/l of carbon in the form of glucose was added. At the end of an additional 12 hour anaerobic digestion period the sludge was elutriated and concentrated. This provided sludge with a phosphorus content between 1 and 1.5 percent.

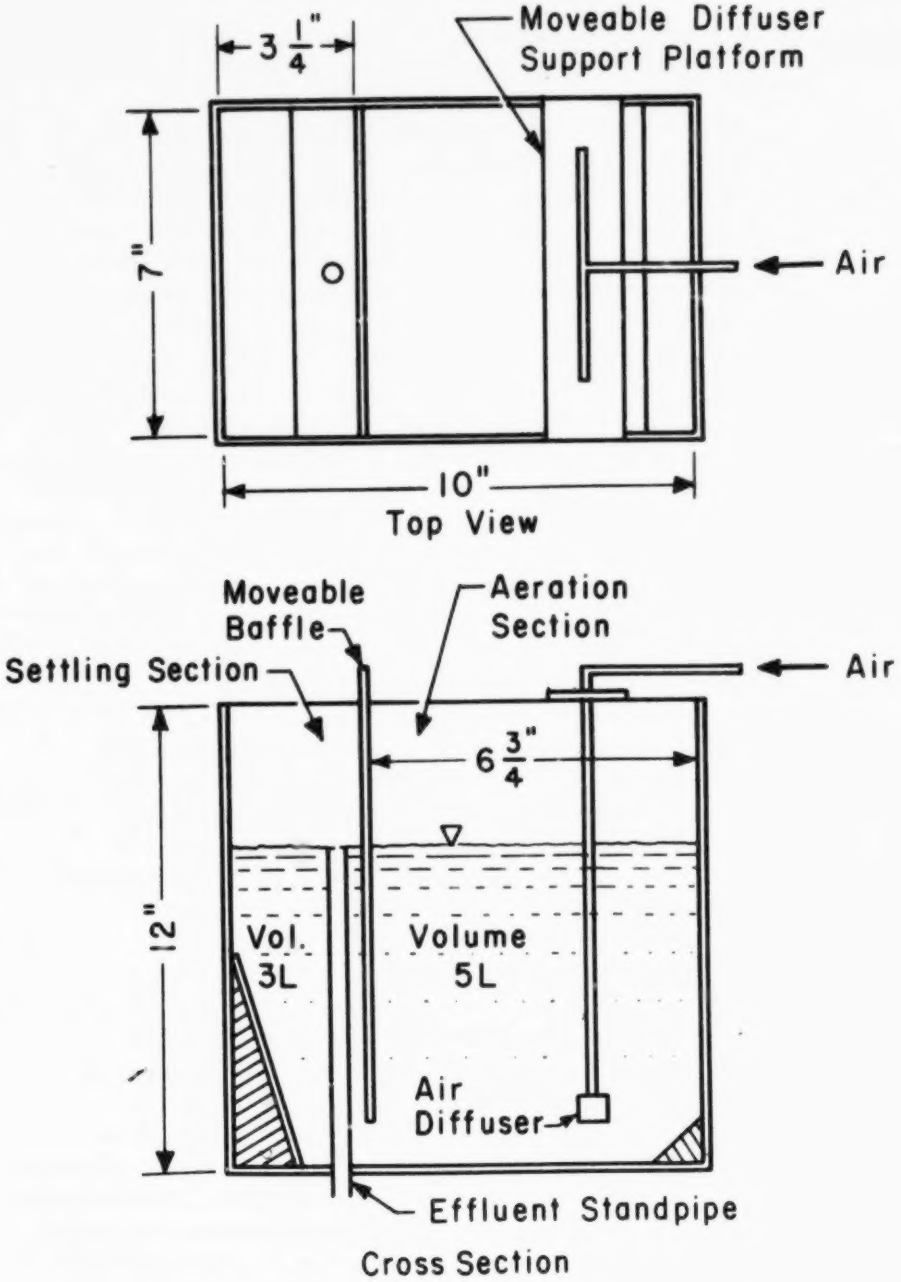
Moveable Diffuser Support Platform
3 1/4"
7"
Air
10"
Top View
Moveable Baffle
Aeration Section
Settling Section
Air
6 3/4"
12"
Vol. 3L
Volume 5L
Air Diffuser
Effluent Standpipe
Cross Section

Figure 9. Sketch of Completely Mixed Continuous Flow Unit.

Synthetic Wastewater Substrates

During the course of the experimental studies several substrates were required (see Appendix B). The substrate used by Higgins (51) during previous studies in this laboratory evaluating the alkaline phosphatase enzymatic assay with batch tests was utilized in the preliminary phases of this study. However, the carbon source was changed from a single source, glucose, to a multi-carbon source to provide a diverse carbon source for the short term (less than one biological solids retention time) completely mixed continuous flow studies. This medium, designated as "Substrate A", provided a suitable substrate for use during the minimum sludge phosphorus content study; i.e., chemical precipitation was avoided as only trace quantities of alkaline or heavy metals necessary for biological metabolism were present. The yeast extract and peptone provided the minimum phosphorus concentration, 0.3 mg/l, during this study. Additional phosphorus as monobasic potassium phosphate was added to obtain higher phosphorus concentrations.

The subsequent short term biological tests combining metabolic and chemical removal of phosphorus required several changes. The carbon concentration was reduced and calcium also was removed from the basic substrate. This medium was designated "Substrate B".

The final biological study was a long term series (greater than 100 days) and a different organic source was used. The multi-carbon source was replaced by glucose, and since minimum phosphorus concentrations were no longer required, the yeast extract concentration was increased and Sego was added to the medium. This medium was designated as "Substrate C".

Automatic pH Control System

During the biological studies it was necessary to maintain a pH range with either a maximum or minimum allowable pH. The control system was composed of two sets of pH electrodes (Corning), a titration pump for each set of electrodes; an electrical circuit that shifted between these sets at six minute intervals; a locally constructed, battery operated expanded scale pH meter; and a recorder (Minneapolis Honeywell) that provided a signal to the titration pump when the preset pH limit was not maintained within the biological unit. The recorder was adjusted to provide a full scale (10 inch) travel for one pH unit. No temperature compensation was provided.

The system pH meter initially was calibrated using a Corning Model 12 pH meter. The electrodes were calibrated using a standard pH 7 buffer solution at least three times daily and at other times when adjustments were made to the units. At regular intervals the probes were cleaned and serviced according to the directions supplied by the manufacturer. Prior to each series of tests the pH meter was calibrated.

To evaluate the performance of the system a 24 hour test was run using chart paper to record the pH variation. With the system set to maintain a maximum pH of 7.05, the maximum pH recorded was 7.05 and the minimum was 6.9. The value below the preset limit was a function of the normality of the titrant and the associated overshoot in titrating to maintain the preset limit. This was controlled in subsequent use by using low normality titrant. Although the results of this evaluation are good, the normal drift of pH electrodes and the occurrence of low battery voltage caused additional drift for short periods upon infrequent occasions. Continuous recording of the pH was not possible due to economic considerations.

Batch Units for Chemical Precipitation Studies

The procedures used for the chemical precipitation tests are described as they occurred during the experimental studies.

Analytical Techniques

The analytical techniques used in this study that are not described in Standard Methods for the Examination of Water and Waste Water, Twelfth Edition (52), are described in this section.

Alkaline Phosphatase Enzymatic Assay

The initial intent was to duplicate the analytical techniques used by Higgins (51) and Moore, et al. (23) to measure the alkaline phosphatase enzymatic activity, thereby maintaining continuity with their batch studies. However, several problems arose from the higher solids content and different characteristics of the sludges used during this study. The changes required to alleviate these problems did not have a significant effect, and are included for evaluation in this respect. The basic technique to measure alkaline phosphatase activity is described below. Following this description the problems encountered during this study are described.

To measure the enzymatic activity a sample was taken from a unit, filtered through a prewashed 0.45 μ membrane filter, placed in a beaker containing 100 milliliters of a p-nitrophenylphosphate (PNP) medium at pH 9.0 (51), and incubated at 35°C. The components of the PNP medium were the same as the synthetic substrate (less the yeast extract and peptone) from which the sample was taken except that the sole source of phosphorus was the PNP. Following various incubation periods the concentration of p-nitrophenol (PN) liberated by the action of alkaline phosphatase was measured with a Bausch and Lomb Spectronic 20 spectrophotometer (wavelength 410 mμ) using a calibration curve of known standards of a p-nitrophenol salt (53). Concurrent with the above, a volatile suspended solids (VSS) measurement was made of the sample and this was used to report the enzymatic activity as μ-moles PN/l per milligram VSS per hour.

During the preliminary experiments the enzymatic activity of continuous flow units could not be replicated. Agglomerated masses of sludge were noted on the filter membrane that had not been present at the lower concentrations used in batch tests. This difficulty was corrected by using a blender at low speed for one minute to disperse the bacteria prior to filtering the sample.

Tests also were carried out to observe the effects of increased solids in the continuous flow units on the enzymatic activity. The data are presented in Table 4. It is apparent that accurate solids measurements (1250 mg/l) could not be attained using sample volumes less than four milliliters. The data in Table 4 also indicate that there is a suppression of the enzymatic activity at higher sample volumes. However, the time from start of filtration to placement of the sample in the incubation chamber also was significantly greater for the larger sample volumes. The effect of filtration time on the enzymatic activity for two different samples is presented in Table 5.

A PNP concentration of 500 mg/l was used during early batch tests, but was increased to 1000 mg/l to avoid any limitation of substrate concentration during the continuous unit tests. As a check that the PNP concentration did not become limiting, random samples were diluted and the enzymatic activity for diluted and undiluted samples was measured (see Table 6). These data indicate that the substrate concentration was adequate, but also indicate that the filtrability of the sludge is more important than the actual quantity of sludge.

This problem was solved by using dilutions when necessary to prevent excessive filtration time. Also, the distilled water washing used

Table 4

Effect of increased Sludge Mass on Enzymatic Activity Using Sludge with 1250 mg/l VSS

Sample Volume (ml)	Sample Mass (mg)	Computed Concentration (mg VSS/l)	Filter Time (min)	Enzymatic Activity (μM/l/mg VSS/hr)
1.0	1.66	1660	2	8.9
2.0	2.79	1395	3	9.1
4.0	5.13	1282	14	8.6
5.0	6.20	1240	23	6.7

Table 5

Effect of Filtration Time on Enzymatic Activity

Test	Filter Time (min)	Enzymatic Activity (μM/l/mg VSS/hr)
1	3	4.2
	23	3.9
	56	3.7
2	3	4.7
	50	4.4
	100	3.8

Table 6

Check Procedure

Sample	Sample Mass (mg)	Filter Time (min)	Enzymatic Activity (μM/l/mg VSS/hr)
Undiluted	9.39	10	7.0
1/2 Dilution	4.70	5	6.6

during the batch tests by Higgins (51) and Moore, et al. (23) was omitted to reduce filtration time.

Phosphorus Measurements

The forms of phosphorus measured were limited to total phosphorus and total soluble phosphorus. Total soluble phosphorus was obtained by first filtering samples through a 0.45 μ membrane filter and subsequent measurement of phosphorus in the filtrate. From these measurements the sludge phosphorus content was computed by subtracting the total soluble phosphorus from the total phosphorus in the aeration tank. This value was used to compute the percent sludge phosphorus content based on volatile suspended solids. Phosphorus removal within the tank was computed by subtracting the total soluble phosphorus in the substrate.

Initially the alkaline ash method reported by Jenkins (56) was used, but difficulties arose with the random occurrence of apparent fusion of phosphorus to the porcelain crucibles used during the ashing procedure. This led to the use of a modified form of the persulfate method of Murphy and Riley (54), which is only slightly different than the method appearing in the FWPCA Methods for Chemical Analyses of Water and Wastes (55) under Phosphorus, Total. Appendix C contains the total phosphorus method used during this study and differences between this method and the above FWPCA method are noted.

Jenkins (57) reported the results of several methods for determination of total phosphorus. Complete recovery was difficult and required drastic digestion procedures, usually a nitric/perchloric or nitric/sulfuric acid digestion yielding the best results. Another method, the alkaline ash, was reported by Jenkins (56) to provide identical results with the nitric/sulfuric acid digestion of the American Association of Soap and Glycerine Producers (58). This alkaline ash method was chosen for recovery comparison with the persulfate method used in this study. The results are shown in Table 7. From these tests a recovery factor of 0.92 was obtained and is applied to the measured sludge phosphorus content during the metabolic uptake experiments.

Other Measurements

Calcium was measured using a Perkin-Elmer Atomic Absorption Spectrophotometer (Model 303). The method described in the Perkin-Elmer

Table 7

Comparison of Alkaline Ash Method to Persulfate Method For Determination of Total Phosphorus

	Persulfate (mg/l P)	Alkaline Ash (mg/l P)
	9.75	10.50
	10.50	11.12
	10.50	11.50
	10.75	11.88
	11.00	11.90
	11.25	12.25
	11.90	12.75
Mean	10.80	11.70
S.D.	0.68	0.73

Recovery Factor $\frac{10.80}{11.70} = 0.92$

Table 8

Precision of Analyses

Analysis	No. of Samples	Minimum	Mean	Maximum	S.D.	Variance (%)
Enzymatic Assay - μM PN/l/mg VSS/hr	9	3.4	4.7	5.6	0.7	15.0
Phosphorus, Total - mg/l	7	9.75	10.81	11.90	0.68	6.3
Phosphorus, Total soluble - mg/l	10	5.72	5.85	5.98	0.07	1.2
Calcium - mg/l	10	70.0	70.8	73.5	1.0	1.4

Analytical Methods for Atomic Absorption Spectrophotometer (59), including the use of lanthanum as a suppressor of anionic interferences, was used.

Suspended solids were measured using glass fiber filters with a Millipore membrane filter apparatus. This method appears in the FWPCA Methods for Chemical Analyses of Water and Wastes (55) under Solids, Nonfilterable. Reeve Angel 934 AH 5.5 cm filters were used.

Soluble total organic carbon (TOC) was measured with a Beckman Total Carbon Analyzer using techniques recommended by the manufacturer. Samples were filtered through a 0.45 μ membrane filter, acidified, and inorganic carbon purged with nitrogen gas.

Fluoride was measured using the Orion Research Fluoride Electrode (Model 94-09) and Orion Research Specific Ion Meter (Model 401). This method appears in the FWPCA Methods for Chemical Analyses of Water and Wastes (55) under Fluoride, Specific Electrode.

Measurements of pH were made with either a Corning Model 12 pH Meter or an Orion Research Specific Ion Meter (Model 401). The pH meters were standardized with buffer solutions prior to each use.

Gravimetric measurements were made using a semi-analytical balance (Mettler Model H16). The readability of this balance is 10^{-5} gram.

Precision of Analyses

The reproducibility of major analytical procedures was determined to establish the precision of a particular analytical technique. These results are presented in Table 8.

SECTION V

METABOLIC UPTAKE OF PHOSPHORUS

This part of the study was conducted to define metabolic uptake of phosphorus by activated sludge systems. To accomplish this, synthetic substrates were developed using concentrations of calcium and phosphorus and a pH range which precluded significant chemical precipitation of phosphorus. Next, it was necessary to define the minimum cellular phosphorus content required for the normal growth rate of bacteria in activated sludge. This minimum cellular phosphorus level was assumed to be the cell phosphorus content at which normal carbon removal (approximately 85-90 percent) from a substrate begins. Also, it was necessary to define the range of cellular phosphorus content above this minimum value in systems that restrict significant phosphorus removal to a metabolic mechanism. As discussed in the literature review, the cellular phosphorus content above this critical level would be defined as "luxury uptake." Concurrently with these experiments the alkaline phosphatase bio-assay was evaluated as a method to detect "luxury uptake" of phosphorus.

Determination of Operating Conditions To Preclude Phosphorus Precipitation

The objective of these experiments was to design a synthetic wastewater in which significant phosphorus precipitation would not occur. However, in developing this substrate, consideration was given to the following:

1) the pH range for good growth of the biomass; and
2) the requirements of the biomass for calcium, magnesium and trace metals.

As discussed in the literature review the laboratory experiments emphasized the calcium-phosphorus system. Control of precipitation was accomplished by restricting the presence and concentrations of cations in the synthetic wastewater substrates to the biologically required quantities.

Although calcium plays a minor role in the growth of microorganisms (24) it was desired to maintain calcium to phosphorus ratios representative of actual domestic wastewaters, between 1.5 and 10 to 1. In the experimental study to define the minimum required sludge phosphorus content for normal growth the concentrations of phosphorus in the substrate would be much smaller (0.01 to 0.15 mM/l) than actual domestic wastewaters (0.25 to 0.5 mM/l). This allowed the calcium concentration of the substrate for this study to be reduced to a minimal value, 0.15 mM/l. (See Appendix B Substrate A.) These low concentrations of calcium and phosphorus precluded significant precipitation in the pH range of interest, 6.5 to 8.5.

The experimental study to evaluate the range of sludge phosphorus content beyond the minimum required for normal growth required a higher phosphorus concentration representative of actual domestic wastewaters. This necessitated the experimental determination of calcium and phosphorus concentrations and the pH range such that significant precipitation of calcium-phosphorus compounds did not occur, yet provided a substrate representative of actual wastewaters.

The effects of pH, magnesium, trace metals and nucleation sites were all considered. The effects of these components in the synthetic wastewater substrates only were evaluated in defining the pH and concentrations of calcium and phosphorus to preclude significant precipitation of phosphorus.

General Procedures

Chemical precipitation tests were required to determine operating conditions for the metabolic uptake of phosphorus in excess of minimum required sludge phosphorus content study. During this study Substrate B (see Appendix B) was used, and the stock components of this substrate were used to prepare the test solutions for the precipitation tests.

The components for each test were added to two liter erlenmeyer flasks containing distilled water and the final sample volume brought to 1 liter. The pH was immediately adjusted to the desired value using sulfuric acid or sodium hydroxide. The flasks were not sealed to the atmosphere, but were cotton plugged to prevent the entry of foreign matter into the solution. All calcium and phosphorus concentrations, including initial values were measured analytically as described in Chapter 4, Experimental Procedures.

The basic time period of these tests was 24 hours; however, several tests were continued for longer periods.

Calcium-Phosphorus Systems

A series of tests were conducted to induce significant precipitation of calcium-phosphorus compounds. This was accomplished using relatively high calcium and phosphorus concentrations compared to concentrations normally encountered in domestic wastewaters. This was necessary to cause significant precipitation and still remain within the pH range of biological systems.

Two tests were run using 10^{-3} M phosphorus with 10^{-2} M and 10^{-3}M calcium at pH 8.5. With the exception of magnesium, bicarbonate, and nitrogen, the solutions contained the inorganic components and EDTA as used in Substrate B. The soluble calcium and phosphorus concentrations remaining in solution were measured after one day and the results are presented in Table 9. As expected the 10:1 Ca_T/P_T ratio precipitated essentially all the phosphorus, and the 1:1 Ca_T/P_T ratio precipitated approximately half of the calcium and phosphorus.

The next series of tests were conducted using 0.1 mM and 0.33 mM phosphorus added to 0.5 mM and 1.0 mM calcium at pH 6.5, 7.25, and 8.0. No other inorganic components of the substrate were added in this series to remove any complexing effects and to reduce the ionic strength to a minimum. The flasks were agitated intermittently during the first 24 hours and left quiescent for 22 additional days. The results of the 0.33 mM phosphorus and the 1.0 mM calcium concentrations after 1 and 23 days are presented in Table 10. No significant phosphorus removal occurred in any test. All of these solutions exceed the theoretical solubility product for hydroxyapatite and the theoretical solubility product for the dicalcium phosphate is exceeded at pH 7.25 and 8.0 in solutions containing 1.0 mM calcium.

The failure of phosphorus to precipitate at the lower pH's prompted a similar series using only 0.33 mM phosphorus and 1.0 mM calcium and including a test at pH 8.5. The presence of small concentrations of inorganic components may exert an effect on the solubility of phosphorus. These potential effects were included in this series by duplicate solutions containing the trace elements present in the substrate. The concentrations of the components were small: boron 2×10^{-4} M, zinc 3×10^{-5} M, manganese 7×10^{-6} M, molybdenum 5×10^{-6} M, copper 6×10^{-6} M, and cobalt 2×10^{-6} M. The flasks were agitated intermittently during the first 24 hours and left quiescent for 3 additional days of the test. The results after 1 and 4 days are presented in Tables 11 and 12. Again there was no significant precipitation at pH 8 or lower. Precipitation did occur in the solutions at

Table 9

Calcium-Phosphorus Systems in Inorganic Medium Less Magnesium, Ammonia, and Bicarbonate

Day	pH	P Total	P Soluble	P Precip.	Ca Total	Ca Soluble	Ca Precip.	Ca:P Precipitate
0	8.5	0.976			1.003			
1		0.937	0.412	0.525	1.000	0.413	0.587	1.12
0	8.5	0.937			9.850			
1		0.995	0.033	0.962	9.750	8.280	1.470	1.53

(Concentrations in millimoles per liter.)

Table 10

Calcium-Phosphorus Systems in Distilled Water
(add pH 6.5, 7.3, and 8.0)

Day	pH	P Total	P Soluble	P Precip.	Ca Total	Ca Soluble	Ca Precip.
0	6.5	0.355			0.956		
1	6.3	0.371	0.355	-	1.000	0.988	-
23		0.361	0.361	-	0.988	0.988	-
0	7.3	0.322			0.949		
1	7.0	0.336	0.322	-	0.962	0.912	-
23		0.329	0.336	-	0.949	0.937	-
0	8.0	0.329			0.921		
1	7.7	0.332	0.332	-	0.926	0.921	-
23		0.329	0.329	-	0.934	0.913	-

(Concentrations in millimoles per liter.)

Table 11

Calcium-Phosphorus Systems in Distilled Water
(add pH 6.6, 7.4, 8.0, 8.5)

Day	pH	P Total	P Soluble	P Precip.	Ca Total	Ca Soluble	Ca Precip.	Ca:P Precipitate
0	6.6	0.319			1.038			
1	6.7	0.313	0.300	-	1.019	0.988	-	-
4		0.326	0.300	-	1.041	1.000	-	-
0	7.4	0.319			1.038			
1	7.5	0.313	0.313	-	1.000	0.975	-	-
4		0.319	0.313	-	1.000	0.962	-	-
0	8.0	0.309			0.975			
1	8.0	0.309	0.313	-	1.000	0.958	-	-
4		0.319	0.306	-	0.988	0.962	-	-
0	8.5	0.294			1.000			
1	8.1	0.319	0.110	0.209	1.000	0.687	0.313	1.50
4		0.355	0.077	0.278	0.988	0.588	0.400	1.44

(Concentrations in millimoles per liter.)

Table 12

Calcium-Phosphorus Systems With Trace Elements

Day	pH	P Total	P Soluble	P Precip.	Ca Total	Ca Soluble	Ca Precip.	Ca:P Precipitate
0	6.6	0.326			0.520			
1	6.8	0.319	0.313	-	0.520	0.520	-	-
4		0.326	0.326	-	0.487	0.475	-	-
0	7.4	0.319			1.023			
1	7.6	0.303	0.294	-	0.988	0.962	-	-
4		0.329	0.311	-	0.988	0.962	-	-
0	8.0	0.309			1.423			
1	8.1	0.306	0.294	-	1.396	1.324	-	-
4		0.300	0.303	-	1.430	1.353	-	-
0	8.5	0.313			1.038			
1	8.2	0.313	0.206	0.107	0.980	0.812	0.168	1.57
4		0.291	0.139	0.152	1.000	0.700	0.300	1.97

(Concentrations in millimoles per liter.)

pH 8.5, both with and without the trace elements present; however, the solutions with the trace elements did exhibit an increase in phosphorus solubility.

Effect of Magnesium

Ferguson and McCarty (35) reported that magnesium exerts a significant effect in calcium-phosphorus systems, increasing the phosphorus solubility between pH 7 and 9. They reported that the effect of magnesium as a minor component requires a Ca:Mg ratio greater than 1 and vanishes as the ratio exceeds 50. Such an effect is of interest in that this pH range is the same as biological systems and the Ca:Mg ratio in many activated sludge systems fall in the required range.

In general synthetic waste substrates contain magnesium concentrations from 0.75 to 1.0 mM. If 1 mM of magnesium is used, 1 mM of calcium would provide the required Ca:Mg ratio reported by Ferguson and McCarty and the requirement of a biological substrate. Also, this concentration of calcium with 0.33 mM of phosphorus has been used in previous tests and no significant precipitation occurred at pH 8 or lower, but did occur at pH 8.5. To observe the possible effect of magnesium at pH 8.5 a test was conducted using these concentrations of calcium, phosphorus, and magnesium at pH 6.5, 7.4, and 8.5. No other inorganic components of the biological substrate were included (but 3 mg/l copper was added as an algicide). The flasks were agitated intermittently for 24 hours. The pH 8.5 test was left quiescent for an additional 17 days.

The results after 1 day are presented in Table 13. A precipitation of phosphorus in the flask with magnesium occured at pH 8.5, but the precipitate was small compared to the test without magnesium. After 18 days no further increase in the precipitated phosphorus occurred. The test without magnesium precipitated approximatley 85 percent of the phosphorus in 4 days, but the presence of magnesium reduced this to approximately 20 percent. These results indicate that magnesium increased the solubility of phosphorus at pH 8.5.

Nucleation Sites

All test series have indicated that significant precipitation of calcium-phosphorus without nucleation sites present did not occur with 10^{-3} M calcium and 3.3×10^{-4} M phosphorus concentrations at pH 8 or lower. Although nucleation sites are not to be included in the substrate, there is the probability that such sites may inadvertently be included

Table 13

Calcium-Phosphorus Systems With One Millimole Per Liter Magnesium

Day	pH	P Total	P Soluble	P Precip.	Ca Total	Ca Soluble	Ca Precip.	Ca:P Precipitate
0	6.5	0.326			1.030			
1		0.326	0.326	-	1.050	1.045	-	-
0	7.4	0.334			1.003			
1		0.329	0.329	-	1.045	1.038	-	-
0	8.5	0.334			1.019			
1		0.321	0.255	(0.066)	1.038	0.997	(0.041)	(0.710)
18		0.336	0.258	(0.078)	-	0.800	(0.230)	(3.000)

(Concentrations in millimoles per liter.)

Table 14

Effect of Nucleation Sites (8 mg/l Kaolinite) on Calcium-Phosphorus Systems With and Without Magnesium, 0.8 mM/l, Containing All Components of the Biological Substrate

Day	pH	Mg	Kaolinite	P Total	P Soluble	P Precip.	Ca Total	Ca Soluble	Ca Precip.
0	8.2	No	Yes	0.316	0.313	-	0.990	0.975	-
1	8.1			0.322	0.306	-	0.988	0.975	-
0	8.2	No	No	0.322	0.313	-	1.000	0.980	-
1	8.1			0.316	0.313	-	1.000	1.000	-
0	8.2	Yes	Yes	0.322	0.322	-	1.000	0.975	-
1	8.2			0.316	0.313	-	1.023	1.005	-
0	8.2	Yes	No	0.300	0.291	-	0.980	0.975	-
1	8.3			0.303	0.300	-	1.030	0.975	-

(Concentrations in millimoles per liter.)

in the inoculum taken from the treatment plant. Should this occur the quantity would be small and their size would be near or in the colloidal range. One of the many likely sources would be some form of clay.

To investigate the effect of such an occurrence a series of tests were conducted with 8 mg/l of kaolinite present in the solutions. The solutions contained all the components of Substrate B, but magnesium was not included in a duplicate set. Duplicates also were run with no kaolinite present. The pH was adjusted to 8.2, a value between observed precipitation at 8.5 and no precipitation at 8.0. Mechanical mixing of the solutions was accomplished using magnetic stirrers. To preclude the possibility of biological growth the flasks were intermittently exposed to ultraviolet light. Other procedures were not changed.

The results of these tests are presented in Table 14. No significant removal of phosphorus occurred in 1 day.

Synopsis

The objective of the tests was to define operating conditions for biological systems with respect to calcium and phosphorus concentrations and the pH range where chemical removal of phosphorus would not be significant. The substrates with 1 mM/l calcium and 0.33 mM/l phosphorus concentrations at pH 8.0 or lower did not cause chemical precipitation of phosphorus. The presence of inorganic components of the biological substrate increased the solubility of phosphorus, particularly magnesium which had been reported to exert a significant effect in the pH range of interest. Small concentrations of nucleation sites in the form of kaolinite did not induce precipitation of phosphorus at pH 8.2 within one day. Should some nucleation sites inadvertently enter the biological system with the inoculum the effect probably would not be significant.

The operation of activated sludge systems under these conditions will provide a synthetic substrate that is representative of actual wastewaters and provide control of chemical precipitation as a significant phosphorus removal mechanism.

Minimum Required Sludge Phosphorus for Normal Growth

This phase of the study was undertaken to define the minimum required cellular phosphorus for normal growth of bacteria as found in activated sludge systems. The experimental units were completely mixed continuous flow laboratory activated sludge units operated for less than one biological solids retention time (mean cell retention time - days).

General Procedures

Low sludge phosphorus content inoculum was used with initial phosphorus contents between 1 and 1.5 percent. The low sludge phosphorus content inoculum was obtained as described in Section 4. Substrate A provided a carbon concentration of 330 mg/l TOC (900 mg/l COD). Influent COD to phosphorus ratios were from 0.03 to 1.5. Influent calcium was 0.15 mM/l and a buffer of 2.5 mM/l carbonate was included in Substrate A. With an average initial inoculum of 500 mg/l volatile suspended solids (VSS) the initial organic loading was approximately 2 pounds COD per pound VSS per day. No sludge was wasted and as the solids increased this organic loading decreased to less than 1 pound COD per pound VSS per day at the end of the series. The hydraulic residence time was 24 hours. The initial conditions in the continuous units were adjusted to approximately steady-state operating conditions; i.e., nutrient levels were adjusted to provide 50 percent of the inorganic and 10 percent of the organic components of the substrate.

The substrate was modified in the second series of experiments; calcium was removed entirely from the substrate and 10 mg/l phenol was added.

Total organic carbon (TOC) was used to report soluble carbon. The relative removal of carbon from the substrate was the primary parameter defining normal growth, and the simplicity of obtaining TOC data prompted the use of this means to define carbon removal in the experiments.

Results

Figure 10 presents the results of the first test series after 72, 96, and 120 hours of operation. The percent TOC removal did not approach a constant 85 to 90 percent until the percent sludge phosphorus increased approximately 1 percent. Below this point carbon removal was not constant, indicating that phosphorus was limiting. Although phosphorus is not limiting above 1 percent sludge phosphorus content, the effluent does not contain significant phosphorus until the sludge phosphorus content exceeds approximately 1.6 percent. Beyond 1.6 percent sludge phosphorus content, phosphorus appeared in the effluent and the rate of the increase in sludge phosphorus content declined rapidly. The maximum sludge phosphorus content obtained during these experiments was 2.5 percent.

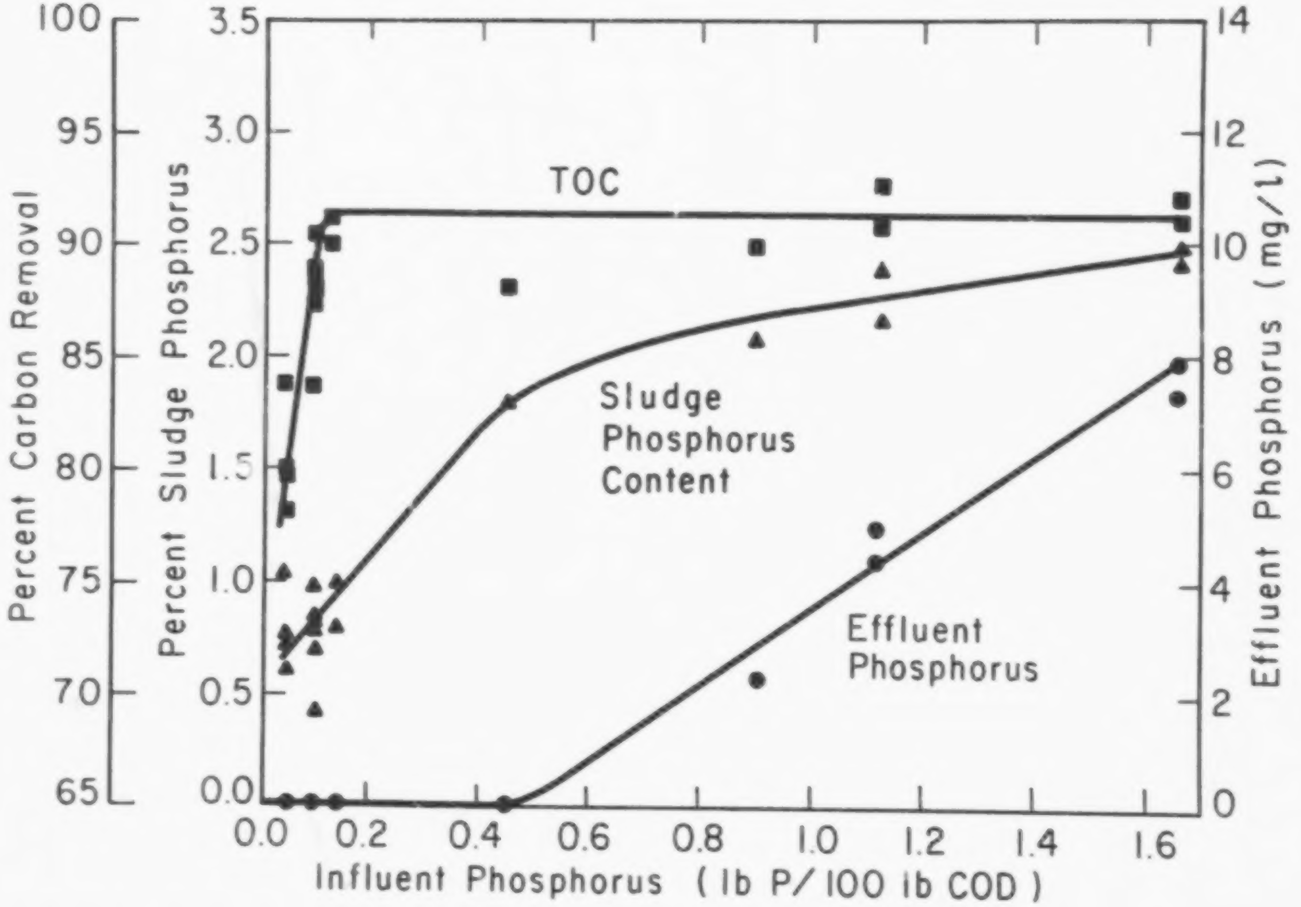

Figure 10. Influent Phosphorus, Effluent Phosphorus, Percent Sludge Phosphorus Content, and Percent Carbon Removal for Laboratory Completely Mixed Continuous Flow Activated Sludge Units (First Test Series).

These results indicate that the minimum cellular phosphorus required for normal growth is between 0.9 and 1 percent. The COD:phosphorus ratio of the substrate at 1 percent sludge phosphorus content is 100:0.15. No significant phosphorus appeared in the effluent until the influent COD:phosphorus ratio exceeded 100:0.45.

The results of the second test series using Substrate A but with no calcium and 10 mg/l phenol after 72 and 96 hours of operation are presented in Figure 11. The relationship between percent sludge phosphorus and TOC removal is essentially the same as that depicted in the first test series. A phosphorus limiting condition occurred below 1 percent sludge phosphorus, and no significant phosphorus appeared in the effluent until the sludge phosphorus content was approximately 1.6 percent. The COD:phosphorus ratios at these points are the same as the first test series, 100:0.15 and 100:0.45, respectively. The maximum sludge phosphorus attained was 1.75 percent.

Synopsis

The results of both test series indicate that the minimum sludge phosphorus content for normal growth, i.e., a constant 85 to 90 percent TOC removal, is between 0.9 and 1 percent. In these short term experiments an influent COD:phosphorus ratio of approximately 100:0.15 was required for this minimum sludge phosphorus content.

Between 1 and 1.6 percent sludge phosphorus content no significant phosphorus appeared in the effluent. The influent COD:phosphorus ratio at 1.6 percent sludge phosphorus content was 100:0.45. Above 1.6 percent sludge phosphorus content, phosphorus appeared in the effluent in both test series.

Although the general relationships between percent sludge phosphorus, TOC removal and effluent phosphorus for the second test series were the same as the first test series, a difference was noted in the maximum percent sludge phosphorus attained. The maximum sludge phosphorus content attained, 2.5 percent, occurred during the first test series at an influent COD:phosphorus ratio of 100:1.63. The maximum sludge phosphorus content during the second test series was 1.75. Above an influent COD:phosphorus ratio of 100:0.6 little increase in sludge phosphorus content occurred up to the maximum ratio of the test series, 100:1.0. This could be attributed to the presence of the phenol, or the lack of calcium in the second test series influent.

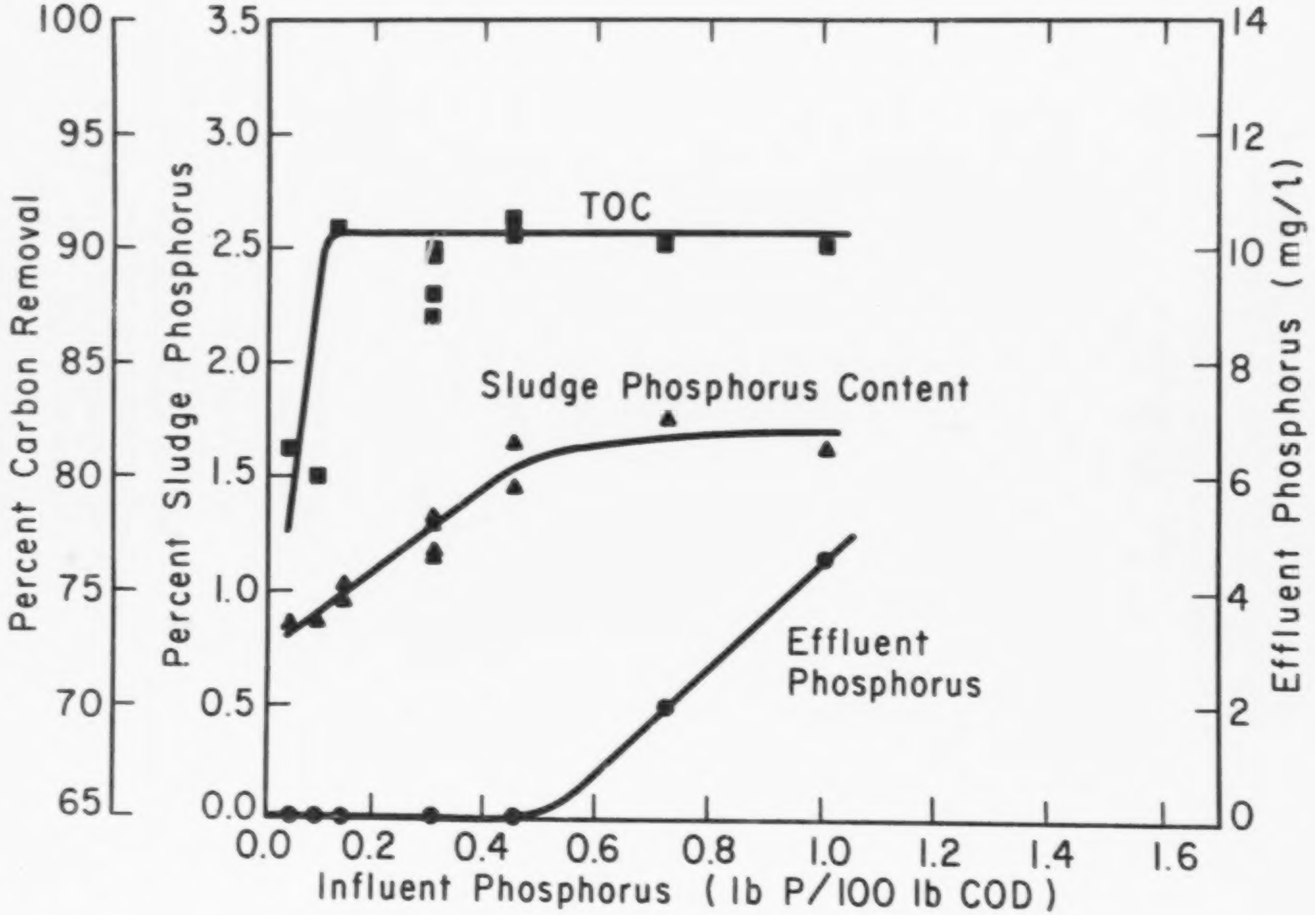

Figure 11. Influent Phosphorus, Effluent Phosphorus, Percent Sludge Phosphorus Content, and Percent Carbon Removal for Laboratory Completely Mixed Continuous Flow Activated Sludge Units (Second Test Series).

However, calcium was being released by the sludge throughout the test; the concentration in the effluent was 8.5 mg/l in the unit with an influent COD:phosphorus ratio of 100:1.0.

Metabolic Incorporation of Phosphorus With Controlled Chemical Precipitation

The sludge phosphorus content of normal activated sludge has been reported to vary between two and three percent. The purpose of this phase of the study was to observe the reaction of sludge phosphorus content in short term completely mixed continuous flow systems inoculated with activated sludges having initial sludge phosphorus contents at the extremes of these limits. Subsequent operation under conditions representative of actual wastewaters, but with significant chemical precipitation controlled as described in the previous section, would indicate the trend of metabolic incorporation of phosphorus. Should the initial high phosphorus content sludge contain any solid phase phosphorus, the controlled conditions should solubilize this phosphorus with a reduction in the sludge phosphorus content. Under controlled conditions any increase in the low initial phosphorus content sludge should reflect only metabolic incorporation of phosphorus.

General Procedures

Inoculum for the high sludge phosphorus content experiment was obtained by using activated sludge as taken from the treatment plant. Only elutriation with distilled water was done to remove nutrients and soluble calcium and phosphorus. The initial sludge phosphorus content was approximately 3.4 percent. The low sludge phosphorus content inoculum was obtained as described in Chapter 4, to provide an initial sludge phosphorus content of approximately 2.0 percent.

Substrate B provided a carbon concentration of 350 mg/l COD (130 mg/l TOC). Sludge was wasted to maintain an organic loading between 0.3 and 0.4 pounds COD per pound VSS per day with a hydraulic residence time of 24 hours. The initial conditions in the continuous units were adjusted to approximately steady-state operating conditions; i.e., nutrient levels were adjusted to provide 50 percent of the inorganic and 10 percent of the organic components of the substrate. TOC again was used to measure carbon removal.

Initial High Sludge Phosphorus Content Inoculum

This series of experiments involved 9 continuous flow units with three having identical influent phosphorus concentrations. Influent concentrations of 5, 7.5, and 10 mg/l phosphorus were used providing COD: phosphorus ratios greater than 100:1 for all substrates. Two of the three units were controlled at pH 7 and the third unit allowed to seek its own operating pH. One controlled pH unit and the uncontrolled pH unit received 40 mg/l calcium in the substrate. The remaining controlled pH unit received no calcium in the substrate.

Table 15 presents the results after 5 days of operation. The effluent TOC of all units indicate normal operation with respect to carbon removal.

The controlled pH units all decreased in sludge phosphorus content. The 7.5 and 10 mg/l phosphorus units were quite close at 2.65 percent and the 5 mg/l somewhat lower at 2.2 percent. The effluent phosphorus concentrations of the controlled pH units were all greater than the influent concentration. Normal carbon removal with excess phosphorus in the effluent indicated that the sludge was slowly releasing phosphorus. The units with no calcium in the substrate did have calcium in the effluent; however, the units with calcium in the substrate did not show an increase in the calcium concentration of the effluent to accompany the increased effluent phosphorus concentration.

The uncontrolled pH units remained above 3 percent sludge phosphorus content with the 5 and 7.5 mg/l phosphorus units near the initial value of 3.4 percent. The effluent of these two units showed a small reduction in the influent phosphorus concentration. The 10 mg/l phosphorus unit had a lower sludge phosphorus content, 3.1 percent, and the effluent phosphorus concentration exceeded that of the influent. Again the calcium concentration in the effluent was essentially the same as the influent.

After the 5 days of operation the 5 mg/l phosphorus units were reversed in the pH control, i.e., the uncontrolled pH unit was controlled at pH 7 and the previously controlled units were uncontrolled. After an additional 5 days of operation the initially controlled pH unit with no calcium in the influent showed no significant change in sludge phosphorus content. The previously controlled pH unit with calcium showed a small increase in sludge phosphorus content, 2.2 to 2.6 percent. The initially uncontrolled unit with calcium did change significantly in sludge phosphorus content. This unit dropped from 3.4 to 1.7 percent

Table 15

Initial High Phosphorus Sludge Content After 5 Days Of Operation With Controlled And Uncontrolled pH

pH Control	Influent P, mg/l	Effluent P, mg/l	% Sludge P	Influent Ca, mg/l	Effluent Ca, mg/l	VSS mg/l	% VSS	Effluent TOC	pH
Yes	5	5.28	2.17	40	38.0	907	86	20	7.0
Yes	5	5.82	2.24	0	5.4	959	86	20	7.0
No	5	4.72	3.43	40	39.0	1162	78	18	7.7
Yes	7.5	9.00	2.68	40	40.7	1057	89	27	7.0
Yes	7.5	10.00	2.65	0	8.4	1131	89	23	7.0
No	7.5	7.12	3.35	40	38.2	1083	79	21	7.7
Yes	10	11.35	2.64	40	38.2	920	89	14	7.0
Yes	10	11.35	2.67	0	6.0	920	90	15	7.0
No	10	11.35	3.12	40	34.4	1010	81	14	7.7

(After 5 days of operation pH control of 5 mg/l influent phosphorus units was reversed. After an additional 5 days, the below data was taken.)

pH Control	Influent P, mg/l	Effluent P, mg/l	% Sludge P	Influent Ca, mg/l	Effluent Ca, mg/l	VSS mg/l	% VSS	Effluent TOC	pH
No	5	4.50	2.59	40	39.5	1050	86	22	7.6
No	5	4.65	2.19	0	3.1	1160	88	22	7.9
Yes	5	6.40	1.69	40	52.5	1318	87	23	7.0

sludge phosphorus content. This was accompanied by an increase in the effluent phosphorus content, 4.7 to 6.4 mg/l, and a large increase in the effluent calcium concentration, 39.0 to 52.5 mg/l. Table 15 presents the results of this pH reversal experiment.

Initial Low Sludge Phosphorus Content Inoculum

This series of experiments were similar to the initial high sludge phosphorus content previously run, but used inoculum that was low in phosphorus content. During the previous series no significant differences were noted with the different influent phosphorus concentrations, or the presence or absence of calcium in the substrate. Therefore, this series used only two units with the 10 mg/l phosphorus and 40 mg/l calcium substrate. To maintain correlation between the series two units with initial high sludge phosphorus content were included that duplicated the 10 mg/l phosphorus and 40 mg/l calcium units from the previous series. As in the previous series one continuou unit of each set was pH controlled and the other unit allowed to seek its own operating pH. No other changes to the general procedures were made.

The results of this series of experiments are presented in Table 16. The effluent TOC values for all units indicated normal carbon removal during the experiments.

The controlled and uncontrolled pH units with initial low sludge phosphorus content were not significantly different in sludge phosphorus content after 5 days of operation, 2.35 and 2.55 percent respectively. These values are close to those found in the previous series, and show an increase in sludge phosphorus content form the initial 2 percent. Phosphorus removal from the influent was occurring in both units.

However, unusual results were noted in the effluent calcium concentrations. The uncontrolled pH unit did not show any change in calcium within the unit, effluent calcium was equal to influent calcium. But, the controlled pH unit had a high effluent calcium concentration, approximately 40 percent above the influent concentration. This release of calcium from the sludge was not balanced by phosphorus. Even though the inoculum was subjected to anaerobic treatment to reduce the phosphorus content, it is apparent that this treatment did not remove all the solid forms of calcium.

Table 16

Initial Low and High Sludge Phosphorus Inoculum; 10 mg/l Phosphorus and 40 mg/l Calcium Influent Concentration

Unit Description	Day	Effluent P, mg/l	% Sludge P	Effluent Ca, mg/l	VSS mg/l	% VSS	Effluent TOC	pH
Controlled pH; Low Phosphorus Sludge	5	8.75	2.57	57.5	989	81	19	7.0
	6	10.00	2.35	55.0	1028	84	20	7.0
	7	10.12	2.47	55.0	1042	82	20	7.2
	8	10.00	2.28	42.5	977	83	20	7.1
	9	9.25	2.85	32.0	878	84	24	7.1
Uncontrolled pH; Low Phosphorus Sludge	5	8.88	2.36	40.5	996	76	19	7.6
Controlled pH; High Phosphorus Sludge	5	10.75	3.44	54.0	945	83	19	7.0
	6	12.00	2.96	48.5	1023	86	20	7.0
	7	14.00	2.31	46.0	1055	87	19	7.0
	8	10.75	2.18	41.0	1070	87	19	7.0
	9	9.50	2.55	40.0	967	89	25	7.0
Uncontrolled pH; High Phosphorus Sludge	5	11.62	3.22	39.0	980	65	20	7.6
	6	12.00	2.93	40.5	1051	75	20	7.5
	7	13.38	2.27	39.0	1107	76	21	7.0
	8	11.12	2.68	49.0	1119	83	20	7.0
	9	10.38	2.46	44.5	984	82	21	7.0

Unusual results also occurred with the initial high sludge phosphorus content units. Both the controlled and uncontrolled units maintained high sludge phosphorus contents with the controlled unit unchanged from the initial conditions, 3.45 percent, and the uncontrolled unit down to 3.2 percent. Excess phosphorus was being released by both sludges.

Again the effluent calcium results are unusual. The uncontrolled pH unit was not releasing calcium to the effluent, yet excess phosphorus was being released. The controlled pH unit was releasing calcium to the effluent, and at a concentration similar to the low initial phosphorus content unit. It appears that solid forms of both calcium and phosphorus were present, but calcium-phosphorus solids were not the sole major form.

Operation of the controlled initial low sludge phosphorus content unit and the two initial high sludge phosphorus units was continued for four additional days. During this period the controlled low initial sludge phosphorus content unit and the uncontrolled high initial sludge phosphorus content unit were subjected to a pH reversal on day 7.

As seen in Table 16 small changes in sludge phosphorus content occurred in the initial low sludge phosphorus unit before and after the pH reversal. The effluent phosphorus concentration indicated phosphorus was being released. The effluent calcium remained above the influent calcium concentration, but decreased after the pH reversal. Day 9 showed the highest sludge phosphorus content, 2.85 percent. This was accompanied by a small removal of phosphorus from the influent, less than 1 mg/l, and a removal of calcium from the influent, 8 mg/l.

The sludge of the controlled initial high phosphorus unit continued to release both calcium and phosphorus through day 8. Effluent phosphorus increased to a high on day 7, then decreased. During this period the sludge phosphorus content decreased to 2.18 percent. On day 9 effluent calcium was the same as the influent and a small removal of phosphorus from the influent occurred. At this time the sludge phosphorus content increased to 2.55 percent.

The sludge of the uncontrolled initial high phosphorus unit continued to release phosphorus through day 6. Before the pH reversal no calcium was released by the sludge. After the pH reversal on day 7 release of calcium occurred, but the release was not as great as either of the previously controlled units in this series. The release of calcium continued through day 9.

Addition of Solid Forms of Calcium and Phosphorus

During the previous series it was noted that solid forms of calcium and phosphorus may have remained within the sludge under uncontrolled pH conditions. To investigate this possibility two uncontrolled pH units were spiked with solid forms of calcium and phosphorus: one with 350 mg/l calcium carbonate and the other unit with 170 mg/l dibasic calcium phosphate. The low initial sludge phosphorus inoculum was used. If these addditions remained in the solid form, sludge phosphorus content in the dibasic calcium phosphate unit and the percent volatile suspended solids for both units would change in relation to the other units. On a basis of 1050 mg/l VSS the solid phosphorus spike (42 mg/l P) would increase the initial sludge phosphorus content by 4 percent. The percent VSS would be decreased approximately 9 percent by the calcium phosphate and 18 percent by the calcium carbonate based on a "normal" percent VSS of 84 percent.

The results of the uncontrolled low initial sludge phosphorus unit with solid calcium carbonate and dibasic calcium phosphate added are presented in Table 17. The effluent TOC values for all units indicated normal carbon removal during the experiments.

During the 9 days of operation both units removed phosphorus from the influent. No calcium release from the calcium carbonate unit occurred and a small release from the calcium phosphate unit only occurred on day 8. The percent VSS showed little variation in the calcium carbonate unit and only slightly more in the calcium phosphate unit. Both units were apparently holding the added solids.

The sludge phosphorus content of the calcium carbonate unit varied slightly with a minimum of 2.35 and a maximum of 2.9 percent, and this was in the range expected from the previous series. The 2.9 percent sludge phosphorus content occurred on day 9. The calcium phosphate unit exhibited a slow decrease in sludge phosphorus content from a high of 6.25 to a low of 5.15 percent sludge phosphorus on day 8. This decline could be primarily attributed to the daily wasting necessary to maintain a constant VSS. On day 9 the sludge phosphorus content increased to 5.75 percent. Although the effluent calcium decreased, the effluent phosphorus did not indicate an increase in phosphorus uptake concurrent with the increased sludge phosphorus content.

Table 17

Initial Low Sludge Phosphorus With $CaHPO_4$ and $CaCO_3$ Spikes; 10 mg/l Phosphorus and 40 mg/l Calcium Influent Concentrations

Unit Description	Day	Effluent P, mg/l	% Sludge P	Effluent Ca, mg/l	VSS mg/l	% VSS	Effluent TOC	pH
Uncontrolled ph; Low Phosphorus Sludge with 170 mg/l $CaHPO_4$	5	8.75	5.73	39.5	993	76	21	7.8
	6	8.38	5.42	40.0	1080	72	19	-
	7	9.12	5.25	40.0	1097	71	20	7.5
	8	8.88	5.15	48.5	1168	65	20	7.3
	9	9.25	5.75	34.5	1007	72	21	7.2
Uncontrolled pH; Low Phosphorus Sludge with 350 mg/l $CaCO_3$	5	8.75	2.59	40.0	903	60	18	7.6
	6	8.75	-	39.5	-	-	20	-
	7	9.50	2.33	39.0	1131	65	20	7.5
	8	9.50	2.41	39.0	1070	65	19	7.3
	9	9.50	2.90	39.5	917	65	21	7.2

Synopsis

Under operating conditions precluding significant chemical precipitation of phosphorus, the high initial sludge phosphorus units all decreased in sludge phosphorus content. The initial low sludge phosphorus content inoculum units increased in sludge phosphorus from the initial 2 percent, but did not exceed 3 percent. The high sludge phosphorus contents maintained by the uncontrolled units indicate that solid forms of phosphorus may remain within the sludge until slowly removed by wasting to maintain a constant solids content; or, as in the pH reversal with both the 5 mg/l and the 10 mg/l phosphorus units, subjected to a pH that will solubilize the solid forms. The addition of solid calcium carbonate and dibasic calcium phosphate to two experimental units substantiated this, and the solid forms remained in the sludge as the pH slowly decreased to a minimum of 7.2 after 9 days of operation. The concentrations of calcium (1 mM/l) and phosphorus (0.33 mM/l) do exceed the theoretical solubility product for dibasic calcium phosphate at this pH. These results indicate that activated sludge can hold solid forms of phosphorus at pH's encountered in many activated sludge plants.

Because of the disparity of correlation between the leaching of phosphorus and calcium from the sludges during these experiments, the high sludge phosphorus contents cannot be assigned to a solid form of calcium-phosphorus being slowly released to solution. Other cations present originally in the inoculum from the wastewater treatment plant can hold phosphorus in solid form and may have caused the results in these tests. Long term (in excess of four biological solids retention time) experiments using laboratory activated sludge systems with calcium as the sole significant precipitating cation should be conducted under conditions that both favor and preclude significant chemical precipitation of phosphorus. This should provide a means of defining an upper limit of metabolic incorporation of phosphorus under laboratory conditions.

Evaluation of Alkaline Phosphatase Enzymatic Assay To Detect "Surplus Uptake" of Phosphorus

Fitzgerald and Nelson (25) developed an alkaline phosphatase bio-assa technique for detection of "surplus uptake" of phosphorus by algae. Using the modification of Fruh and Pessoney (26), Higgins (51) and Moore, *et al*. (23) evaluated this technique to detect "surplus uptake" by activated sludge. Their experiments were conducted with batch units. The purpose of this chapter is to present the results of an evaluation of the alkaline phosphatase assay as a method to detect "surplus uptake" of phosphorus using completely mixed continuous flow laboratory activated sludge systems.

During the experimental studies defining the limits of metabolic incorporation of phosphorus by activated sludge, the percent sludge phosphorus content was the primary parameter (coupled with carbon removal by the sludge) used to define the minimum sludge phosphorus requirement for normal growth, and in conjunction with effluent phosphorus was used to evaluate the range of "surplus uptake" of phosphorus. Concurrently with these studies the alkaline phosphatase activity of the activated sludges was measured. This gave a direct comparison of the results from the two methods to evaluate the alkaline phosphatase bio-assay to detect "luxury uptake" of phosphorus.

General Procedures

The procedures used to measure the alkaline phosphatase activity are outlined in Chapter 4.

The enzymatic activity study using completely mixed continuous flow activated sludge units was concurrent with the metabolic studies, and the general operating procedures for the activated sludge units are described in the appropriate sections of those studies. The alkaline phosphatase activity was measured at the same time from samples used to obtain the data presented in the previous sections. The assay medium was the same as the substrate in use during a particular experiment (less yeast extract and peptone).

Inoculum for the batch tests was obtained from the completely mixed continuous flow activated sludge systems used during the metabolic uptake of phosphorus with controlled chemical precipitation study. Although the substrate of the continuous flow units contained 40 mg/l of calcium, the calcium concentration was reduced to 6 mg/l for the batch test substrates to duplicate that of Moore, et al. (23). Also, the pH of the batch units increased during the test period (up to 8.5) and this low calcium concentration was necessary to preclude the possibility of chemical precipitation of phosphorus. The phosphorus concentration varied from 0.2 mg/l to 6.35 mg/l. The time period of the tests was 22 hours.

Results

The relationship between the alkaline phosphatase activity and the percent sludge phosphorus content for the first test series of the minimum required sludge phosphorus content for normal growth study is depicted in Figure 12. The data presented were obtained after 3, 4, and 5 days of operation. The percent sludge phosphorus content data corresponds to that previously presented in Figure 10.

There is an apparent relationship between the enzymatic activity and the percent sludge phosphorus content. At sludge phosphorus contents of 1 percent and lower relatively high enzymatic activities were exhibited by the sludges. This corresponds to the minimum required sludge phosphorus content for normal growth, i.e. below which phosphorus is limiting. No sludge phosphorus contents between 1 and 1.6 percent occurred in this test series, but the results of sludge phosphorus contents above 1.6 percent indicate a relatively constant enzymatic activity. The relationship depicted between 1 and 1.6 percent could be assumed. This assumption would follow the experimental results described in the section defining minimum required sludge phosphorus content, i.e., a transition from a limiting nutrient condition to a condition of available surplus phosphorus.

The results of the second test series of the minimum required sludge phosphorus content study are presented in Figure 13. The only difference between the two test series was in the substrates; calcium was removed and 10 mg/l of phenol was added to the substrates in the second test series. The percent sludge phosphorus content data corresponds to that previously presented in Figure 11. The relationship between enzymatic activity and percent sludge phosphorus from the first test series also is shown.

A significant suppression of the enzymatic activity apparently occurred with the changes in the substrate. As seen in Figure 13 the maximum enzymatic activity measured did not exceed the minimum enzymatic activity measured in the first test series. The minimum enzymatic activities also were correspondingly lower. Although the enzymatic activities are suppressed, the relationship between enzymatic activity and percent sludge phosphorus content between 1 and 1.6 percent is depicted as a transition zone. Such a transition zone assumed in the 1 to 1.6 percent range in the first test series appears to be valid.

Figure 14 presents the results of the metabolic incorporation of phosphorus with controlled chemical precipitation study. Again, the

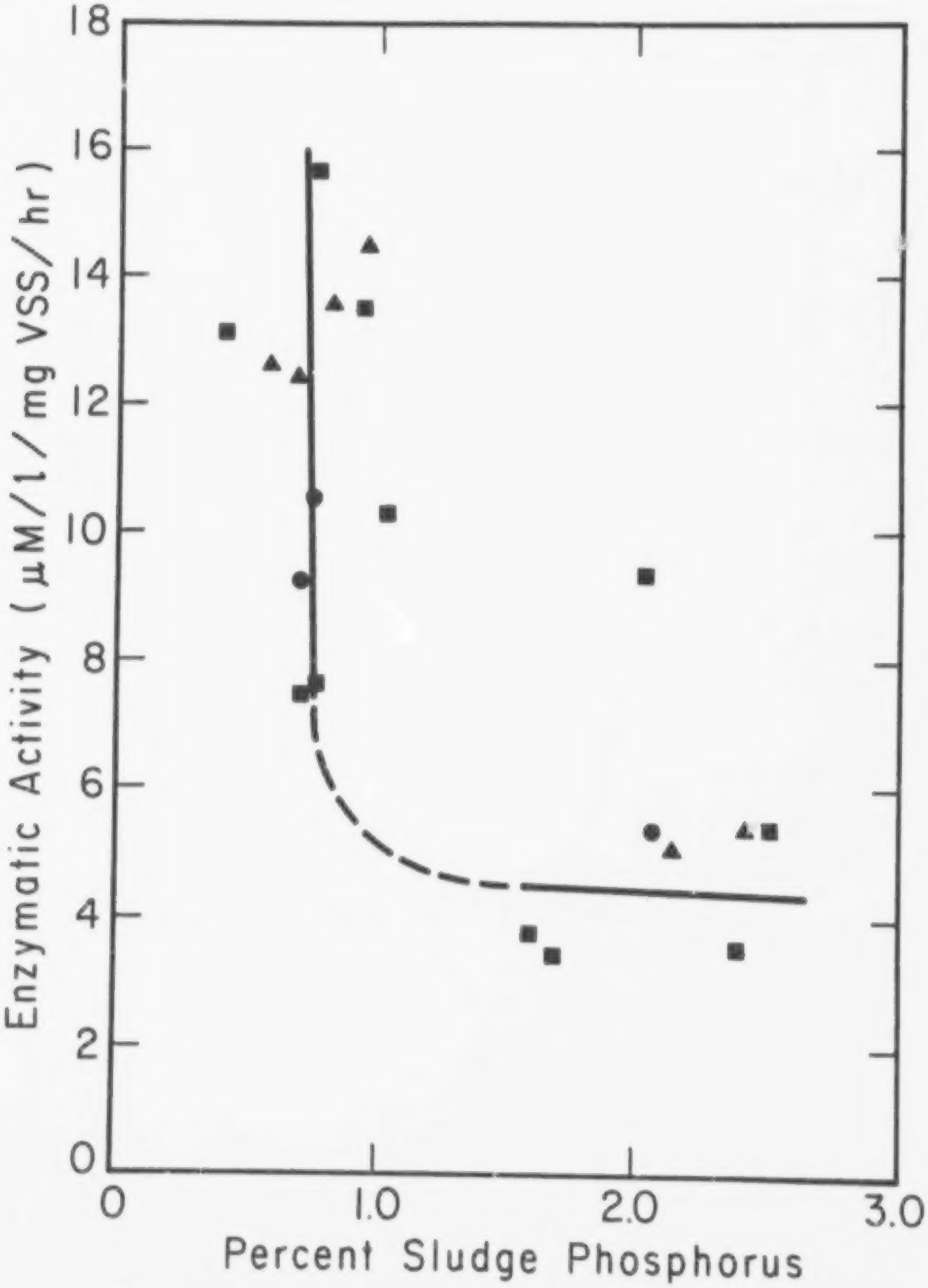

Figure 12. Results of Alkaline Phosphatase Bioassay - Minimum Sludge Phosphorus Content Study, First Test Series.

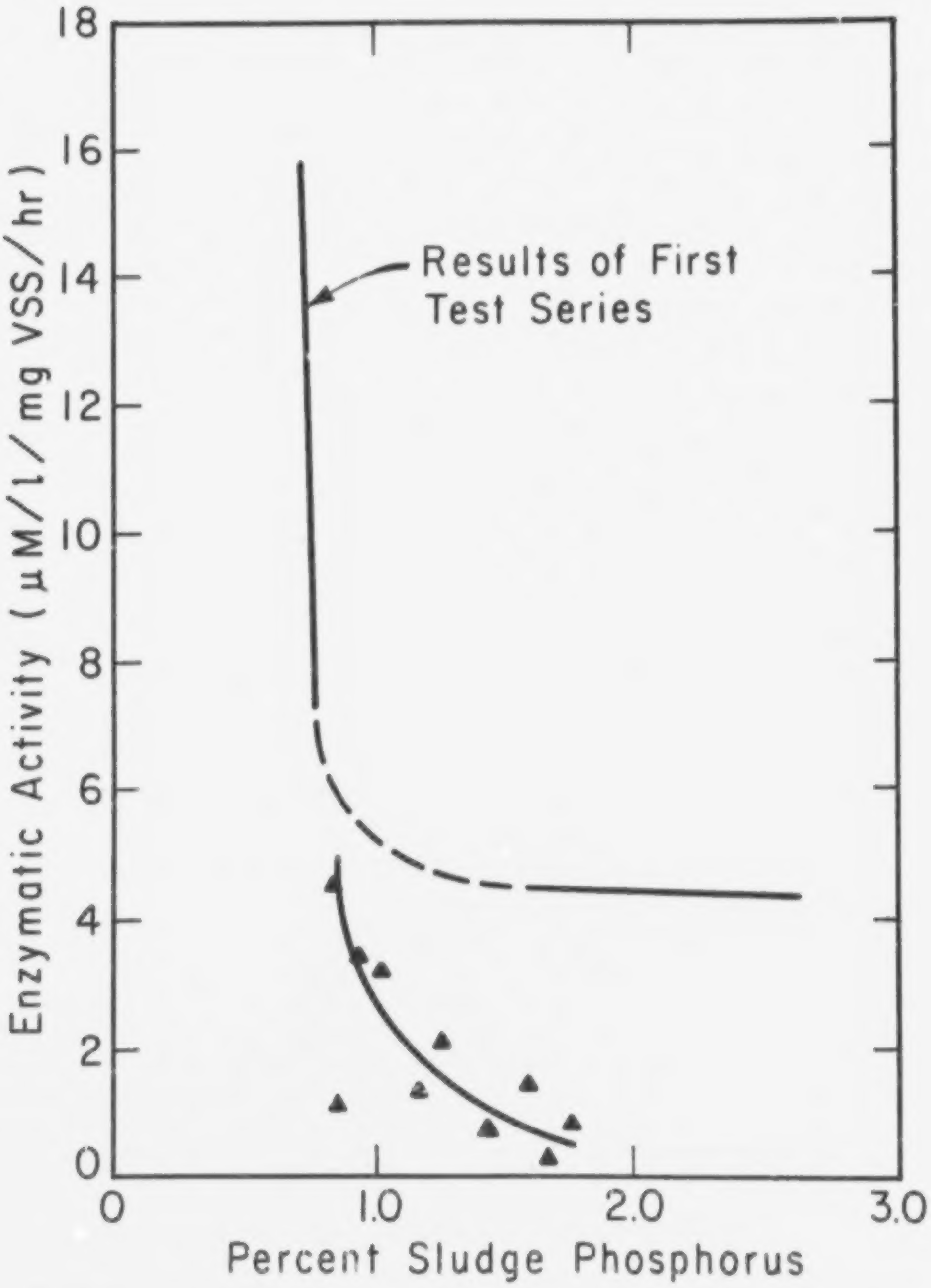

Figure 13. Results of Alkaline Phosphatase Bioassay - Minimum Sludge Phosphorus Content Study, Second Test Series.

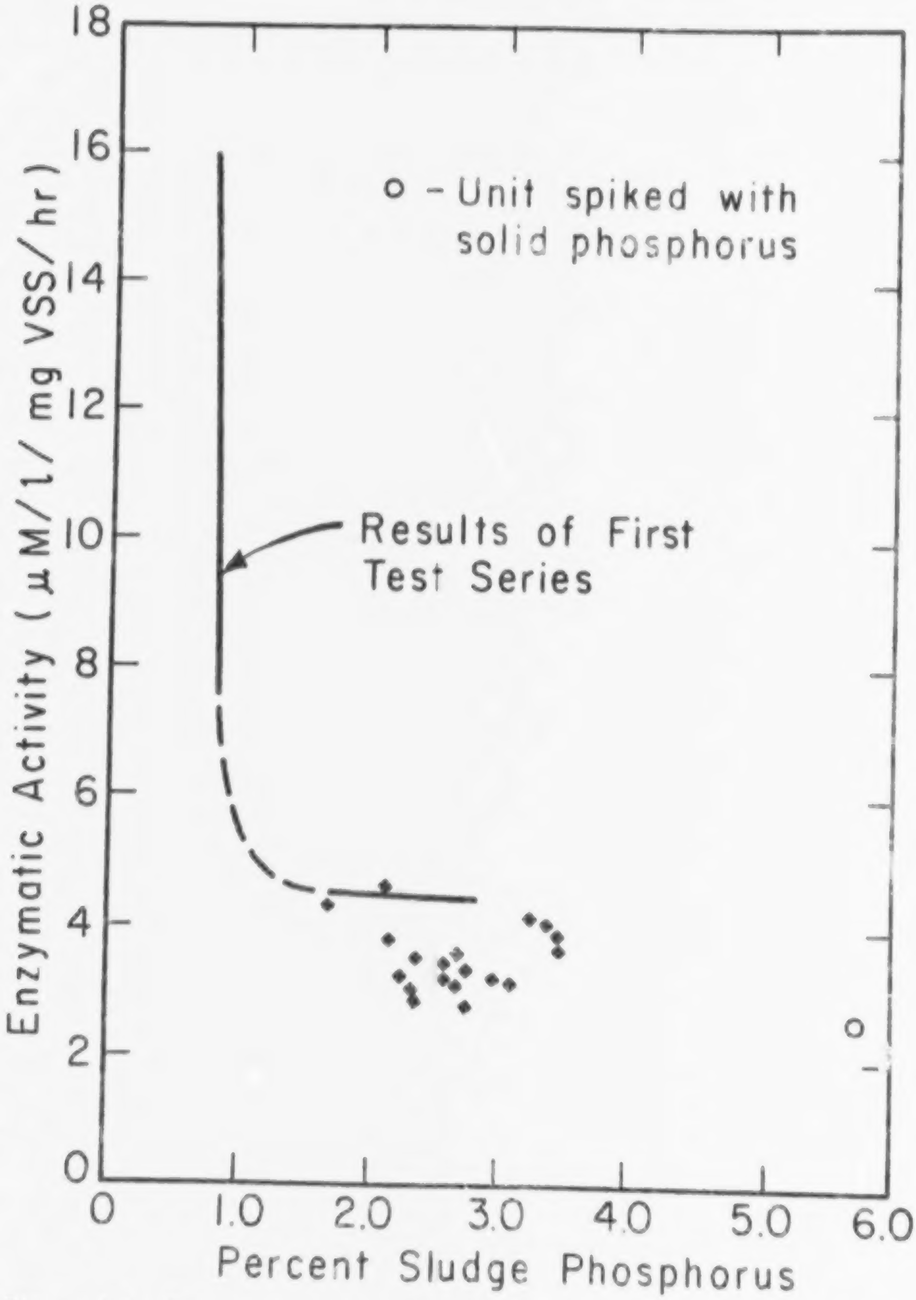

Figure 14. Results of Alkaline Phosphatase Bioassay - Metabolic Incorporation of Phosphorus with Controlled Chemical Precipitation Study.

Table 18

Batch Tests

Initial Conditions				Final Conditions						
TSS mg/1	TOC mg/1	P Soluble mg/1	% Sludge P	TSS mg/1	TOC mg/1	P Soluble mg/1	% Sludge P	Enz. Act.	Δ TSS mg/1	Phosphorus utilized
139	135	0.20	1.73	240	42	0.04	0.99	8.3	101	0.16
140	118	0.27	1.75	231	14	0.02	1.12	8.0	91	0.25
141	118	0.77	1.77	225	15	0.04	1.19	7.8	84	0.73
149	120	0.76	1.78	228	15	0.04	1.28	7.8	79	0.72
137	125	0.72	1.80	234	17	0.04	1.19	7.3	97	0.68
138	135	1.29	1.76	255	25	0.10	1.53	6.0	117	1.19
130	135	2.42	2.12	289	25	0.30	1.67	2.5	159	2.12
128	118	3.12	2.07	247	-	0.67	1.56	2.7	119	2.45
131	135	4.75	2.04	272	25	2.36	1.98	2.5	141	2.39
133	135	6.35	1.76	259	25	4.25	1.95	2.4	126	2.10

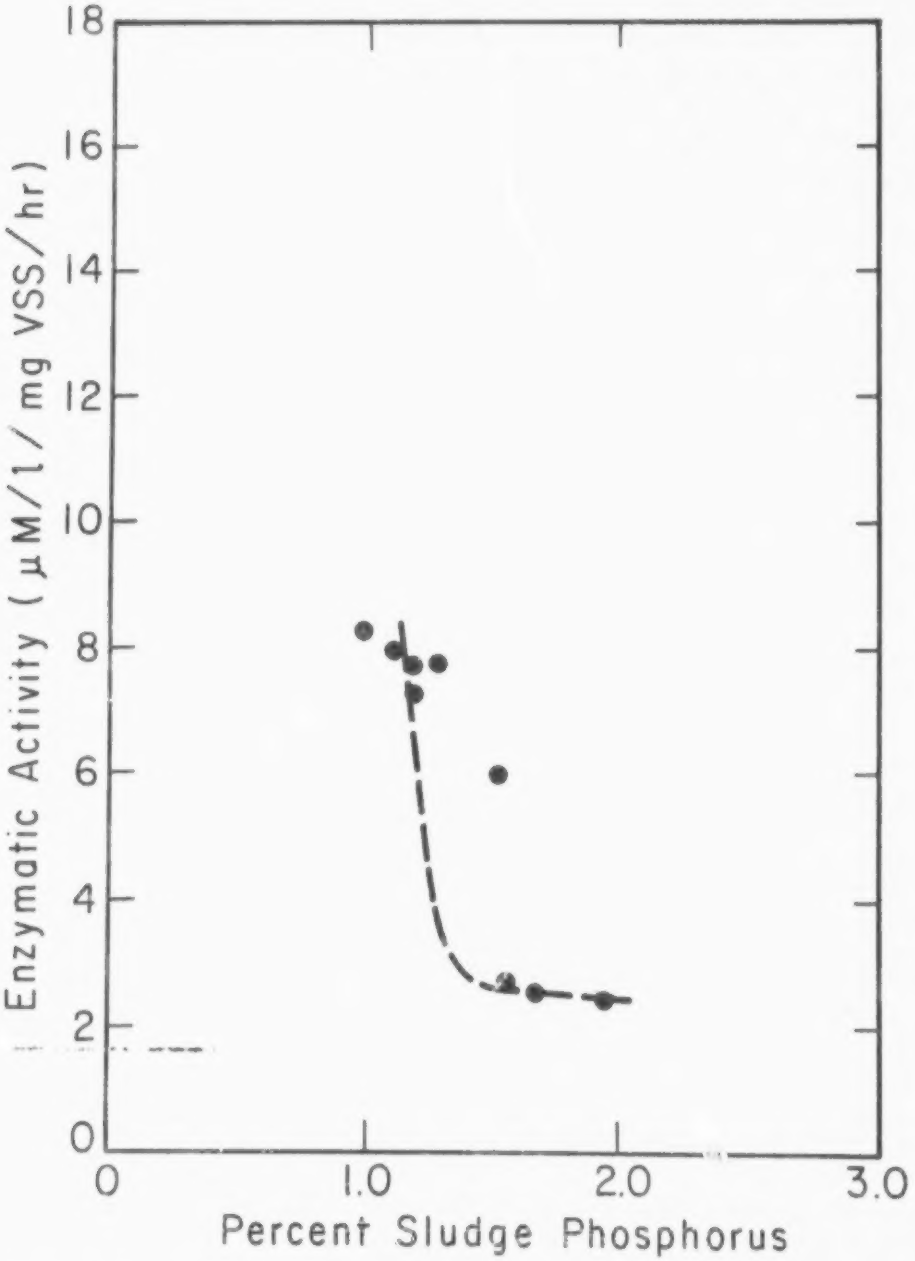

Figure 15. Results of Alkaline Phosphatase Bioassay - Batch Tests.

relationship between enzymatic activity and percent sludge content obatined in the first test series of the minimum sludge phosphorus content study is shown.

These data appear to fit the relationship previously demonstrated. Although the enzymatic activities appear to be slightly lower, a relatively constant enzymatic activity occurred at these higher percent sludge phosphorus contents.

The results of the batch tests are presented in Table 18. Figure 15 depicts the relationship between enzymatic activity and percent sludge phosphorus. Carbon removal was essentially complete in all units indicating that the activated sludges were in, or close to the stationary phase of the growth cycle. As a result no high percent sludge phosphorus contents were obtained similar to the results of Moore, et al. (see Figure 2); the high percent sludge phosphorus contents of their study only occurred during the lag phase of the growth cycle.

The low enzymatic activities in this series occurred at much lower percent sludge phosphorus contents compared to the results of Moore, et al. The results indicate that a much sharper transition occurred in this test series between the high and low enzymatic activities exhibited by the batch units, but still depict a relationship between enzymatic activity and percent sludge phosphorus content similar to the continuous flow tests.

Synopsis

In previous sections of this chapter two zones of percent sludge phosphorus content were defined: the growth dependent zone, less than 1 percent sludge phosphorus; and the storage zone, between 1 and 1.6 percent sludge phosphorus content. The relationship between alkaline phosphatase activity and percent sludge phosphorus content demonstrated in these tests defines similar zones.

In the study on the minimum required sludge phosphorus content for normal growth, phosphorus was shown to be limiting below 1 percent sludge phosphorus content. The enzymatic activities measured in this zone were relatively high, indicating that the microorganisms were exerting a phosphorus demand. Between 1 and 1.6 percent sludge phosphorus, normal growth occurred but all phosphorus in the substrate was utilized. The enzymatic activities measured in this zone depicted a transition from the relatively high enzymatic activities of the phosphorus limiting zone to the relatively constant enzymatic activities obtained for sludges having a phosphorus content above 1.6 percent. Above 1.6 percent sludge phosphorus content, phosphorus appeared in the effluent, and concurrently with this the enzymatic activities approached a relatively low constant value. The higher sludge phosphorus contents of the experimental study with controlled chemical precipitation also demonstrated a relatively constant enzymatic activity above 1.6 percent sludge phosphorus content.

The results of this batch test series generally agree with the results reported by Moore, et al. with respect to the conditions necessary for high enzymatic activities. However, the enzymatic data from the batch tests compared with those from the continuous flow experiments indicate that a quantitative evaluation of phosphorus demand using this technique is not possible.

In previous and current batch tests with similar substrate and environmental conditions, the enzymatic activity was quantitatively related. However, for microorganisms fed with different substrates and cultured under different environmental conditions, the enzymatic activities cannot be quantitatively correlated although each is qualitatively high or low in relation to the sludge phosphorus content within its own test series. For example, for a sludge phosphorus content of 1.75, enzymatic activity ranged from above 4.0 (Figure 14) to less than 1.0 (Figure 13). This variation in enzymatic activity greatly exceeds the variance of the test.

Summary

With a synthetic substrate that precluded chemical precipitation of phosphorus, the minimum required sludge phosphorus content for normal growth, i.e., constant 85 to 90 percent carbon removal, was found to be between 0.9 and 1.0 percent (based on volatile suspended solids) in short term (less than 1 biological solids retention time) completely mixed continuous flow laboratory activated sludge units. Additionally, two zones of sludge phosphorus content for activated sludge were defined:

1) below 1 percent, a growth dependent zone, and,
2) between 1 and 1.6 percent, a storage zone.

Beyond 1.6 percent sludge phosphorus content additional phosphorus was incorporated by the sludge, but at a constantly decreasing rate. The maximum sludge phosphorus content attained was 2.5 percent.

The range of "luxury uptake" of phosphorus during these experiments was 1.5 percent, from 1 to 2.5 percent sludge phosphorus content.

Operating conditions of pH (7.0) and concentrations of calcium (1 mM/l) and phosphorus (0.33 mM/l) were determined to provide a synthetic substrate representative of actual wastewaters that precluded significant chemical precipitation of phosphorus content at the extremes of the "normal" sludge phosphorus content of 2 to 3 percent, the maximum phosphorus content attained in short term completely mixed continuous flow laboratory activated sludge units was less than 3 percent. Under conditions of no pH control (7.8 to 7.2) solid forms of calcium and phosphorus added as calcium carbonate and dibasic calcium phosphate remained within the sludge until removed by sludge wasting.

The alkaline phosphatase enzymatic activity bio-assay was evaluated as a method to detect "luxury uptake" of phosphorus by activated sludge. The results obtained using the enzymatic assay served as substantiating evidence of the defined zones of sludge phosphorus content.

SECTION VI

CONCURRENT CHEMICAL AND METABOLIC INCORPORATION OF PHOSPHORUS

In the preceding section the incorporation of phosphorus in the sludge was restricted to metabolic uptake by operating conditions of pH and concentrations of calcium, phosphorus and other cations such that significant precipitation of phosphorus did not occur. The purpose of the experimental studies reported in this section was to determine the magnitude of phosphorus incorporation in the sludge mass by precipitation of phosphorus with calcium.

To accomplish this it was necessary to define operating conditions representative of actual wastewaters, but such that chemical precipitation of calcium and phosphorus would be induced. Simultaneous operation of completely mixed continuous flow activated sludge systems with operating conditions that favor and preclude significant calcium-phosphorus precipitation would provide a means of separating the quantity of phosphorus incorporation in the sludge by each mechanism.

Additionally, two ions, magnesium and fluoride, have been reported to exert an effect on the calcium-phosphorus system; and, a concurrent investigation of possible effects was included in the experimental studies.

Operating Conditions to Induce Chemical Precipitation

In determining the operating conditions to induce chemical precipitation of phosphorus with calcium, the results of several studies discussed in the literature review, and the operating conditions of some activated sludge plants reporting high phosphorus removals were considered:

1) magnesium exerts an effect on the solubility of phosphorus within the concentration and pH ranges encountered in most activated sludge plants (35,37);
2) there is a kinetic effect at these concentrations and pH's (33,37);
3) fluoride may exert a significant effect on the residual soluble phosphorus (33);
4) the pH range of several activated sludge plants reporting high removals of phosphorus is below 8 (4,27,28); and
5) the concentrations of calcium and phosphorus are less than 2.5 mM and 0.5 mM, respectively, at these plants, and fluoride is present in the influent wastewaters at concentrations between 0.5 and 2.0 mg/l.

These areas of consideration present a relatively large latitude in choosing operating conditions to induce chemical precipitation in calcium-phosphorus systems in activated sludge. However, the choice of conditions was based on the availability of a study by Ferguson, et al. (37).

Ferguson, et al. investigated calcium-phosphorus systems containing 2.0 mM of calcium and 0.24 mM of phosphorus in chemically defined solutions containing only calcium, phosphorus and carbonate at pH's of 7.6 and 8.0. Their results showed significant phosphorus precipitation occurred at both pH 7.6 and 8.0. Although the residual soluble phosphorus was the same at both pH's after 5 days, the presence of an induction period noted was a function of pH.

The effect of varying concentrations of magnesium exerted an effect on the residual soluble phosphorus, increasing the residual soluble phosphorus as the calcium to magnesium ratio decreased to 3 to 1 at pH 8.0. An induction period was noted at a calcium to magnesium ratio of 1.4 to 1, with a further increase in the residual soluble phosphorus.

The concentrations of calcium and phosphorus and the pH ranges in the study of Ferguson, et al. satisfy the limits already described; thus, their results would provide a basis for comparison with the results obtained during this study in solutions containing the components of the biological substrate. Therefore, concentrations of 2.0 mM of calcium and 0.33 mM of phosphorus were chosen for this study. The slightly higher phosphorus concentration maintained continuity with the preceding experimental studies presented in Chapter 5. A pH of 7.6 was chosen to approximate the range of some activated sludge plants reporting unaccounted for high phosphorus removals.

As in the experimental study to determine operating conditions to preclude a significant calcium precipitation, the effects of the components in the synthetic wastewater substrate were evaluated only to define the conditions that induce calcium precipitation of phosphorus. The quantitative effects on the solubility of calcium-phosphorus systems were not investigated.

General Procedures

Batch tests were conducted in 500 milliliter Erlenmeyer flasks with ground glass stoppers. Continuous mechanical mixing was provided by magnetic stirrers. The substrate to be used during the concurrent metabolic and chemical removal study was Substrate C and stock solutions of the components of this substrate were used to prepare the test solutions. Substrate C contained the same ratios of inorganic components used in Substrate B. The buffer system also was composed of 2.5 mM of bicarbonate. Trace elements were present in the same concentrations used in previous continuous flow experiments (see Chapter 5). Sterile techniques were employed to prevent contamination of the test solutions containing the organic component of the substrate.

The pH of the test solutions was initially adjusted to 7.6. The pH was checked twice daily thereafter, and adjusted to this value if required. The pH of the test solution containing the organic component was initially adjusted to 7.6; and, as the flasks were kept sealed during the tests to prevent contamination, no further pH adjustments were made. The flasks containing only inorganic components also were sealed except during pH adjustments.

Experimental Results

Calcium-Phosphorus Systems: The results of a batch test containing only calcium and phosphorus are presented in Figure 16. Tyndall light scattering was observed 8 hours after the start of the test. The presence of solids became visible shortly thereafter, and the turbidity of the solution increased rapidly. At 15 hours, almost half of the phosphorus had been precipitated. Unfortunately, the pH decreased to 6.8 overnight. After adjustment to pH 7.6 the precipitation of phosphorus continued as before and at 41 hours 8.0 mg/l of phosphorus had been precipitated.

Effect of Magnesium: Another batch test contained the basic calcium-phosphorus concentrations but also the concentration of magnesium, 0.8 mM/l (Ca:Mg ratio of 2.5:1), to be used in the substrate during the continuous unit studies. The results are presented in Figure 17.

These results are very similar to those obtained in the calcium-phosphorus only system; the curves are almost identical up to the 41 hour sample. The same pH decrease noted in the calcium-phosphorus only test also occurred during this test. The residual soluble phosphorus concentration was 0.043 mM (1.33 mg/l) after 120 hours.

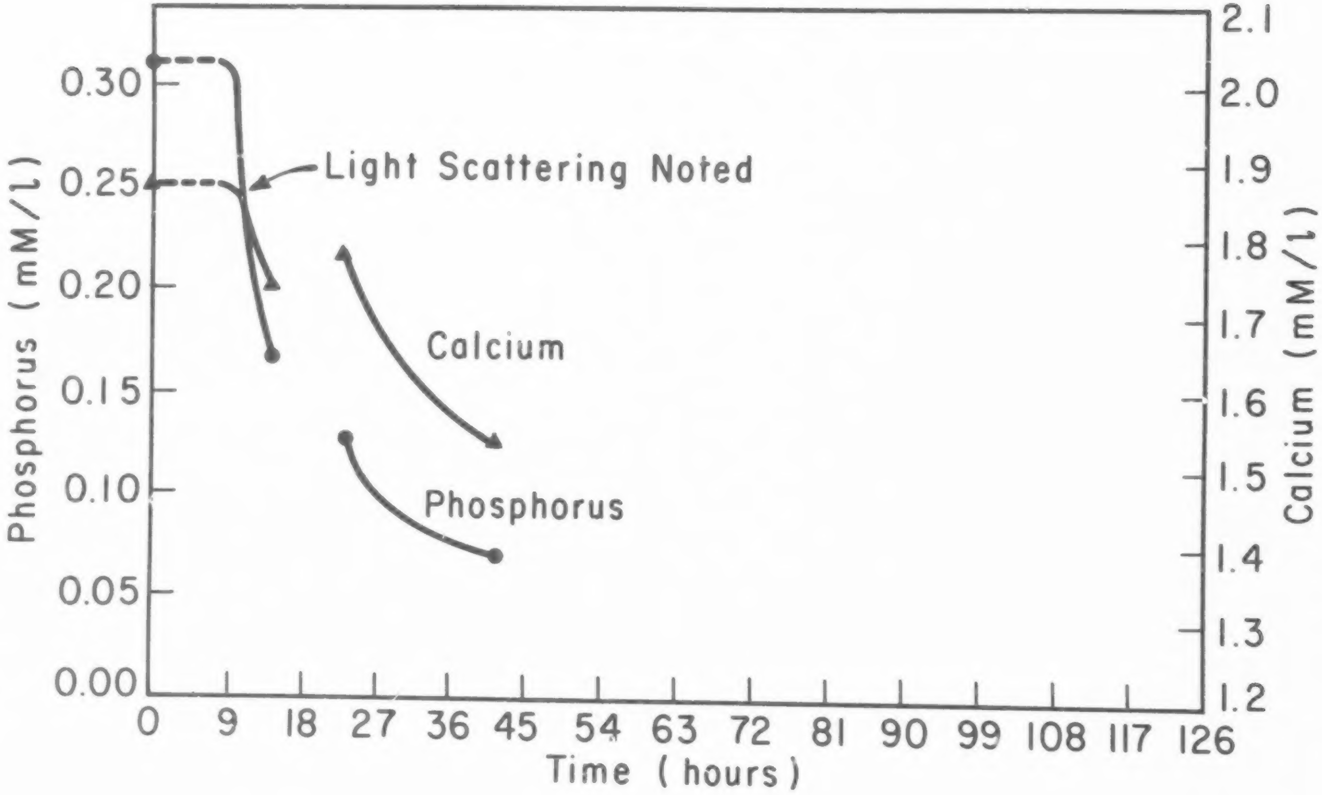

Figure 16. Batch Chemical Precipitation Tests; 2 mM Calcium, and 0.33 mM Phosphorus.

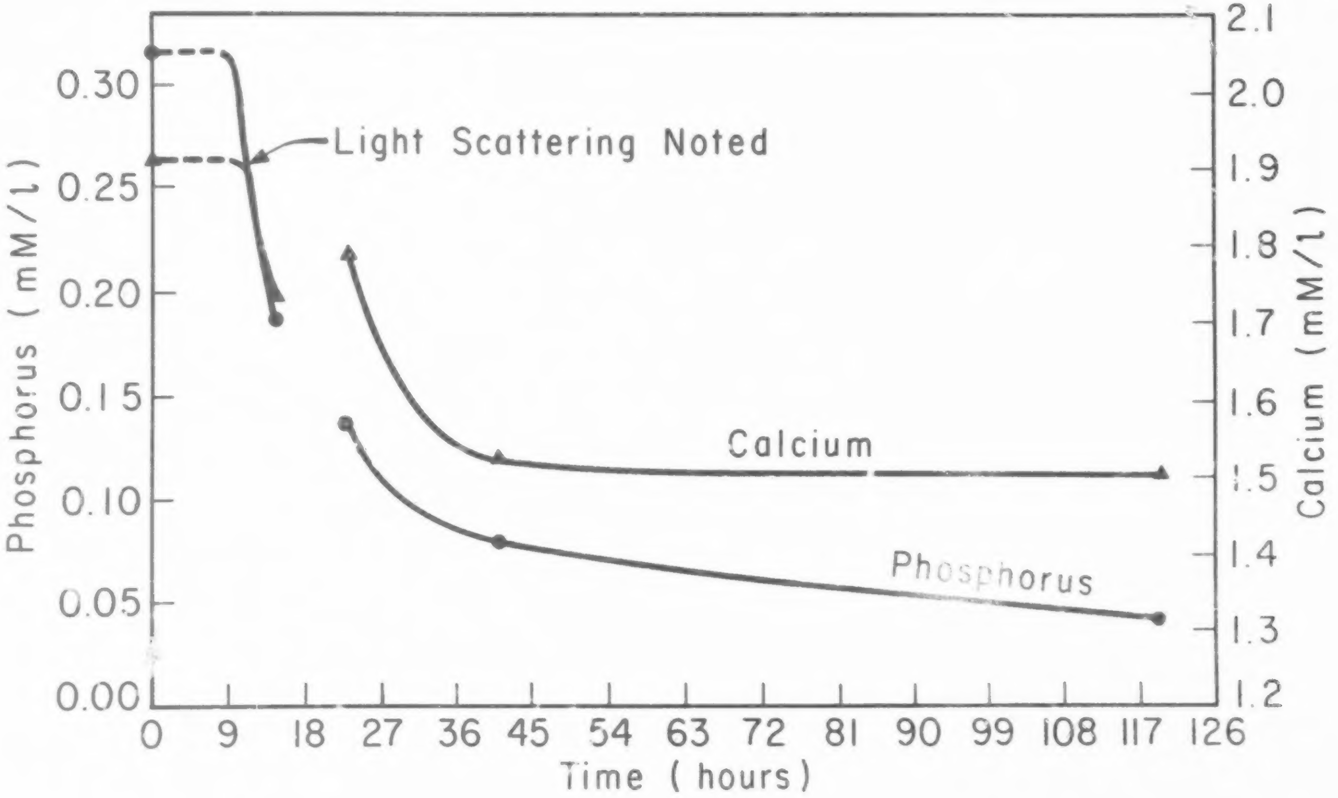

Figure 17. Batch Chemical Precipitation Tests; 2 mM Calcium, 0.33 mM Phosphorus, and 0.8 mM Magnesium.

The results of the next batch test containing a high magnesium concentration, 2.0 mM (Ca:Mg ratio of 1:1), are presented in Table 19. The high magnesium concentration test solutions did not exhibit any measureable reduction in soluble phosphorus after 60 hours.

Effect of Fluoride: A test containing only the basic calcium-phosphorus concentrations with 1 mg/l fluoride added was conducted to observe the effect of fluoride on residual soluble phosphorus. Another test solution containing 1 mg/l fluoride with a Ca:Mg ratio of 1 was also studied. Results are presented in Table 19.

Fluoride did reduce the residual soluble phosphorus compared to the calcium-phosphorus solution with no fluoride. After 27 hours there was a 0.024 mM (0.74 mg/l P) difference between the test solutions with and without fluoride. The fluoride concentration was reduced to less than 0.1 mg/l indicating that fluoride did enter into the reaction. There was approximately a 4 hour increase between the time that light scattering was noted in the unit without fluoride and when light scattering in the fluoride unit was observed.

The test solution containing magnesium and fluoride lost some fluoride, but no calcium or phosphorus. The use of glass flasks and the adsorption of fluoride by glass could account for this small loss.

Effect of Inorganic Substrate Components: The effect of the inorganic components of the substrate, including carbonate but not magnesium, was observed in a batch test. The concentrations of these inorganic components were the same as would be used in the synthetic substrate for the concurrent metabolic and chemical removal study. The EDTA was included in the inorganic stock solution and was present in this test solution. The results are presented in Figure 18.

The results of this test were again similar to the experiment containing only calcium-phosphorus except that a much longer induction period occurred; more than twice the time period previously observed. The residual soluble phosphorus was slightly lower, 0.032 mM (1.0 mg/l). The presence of the inorganic components apparently exerted only a kinetic effect, with little effect on the final residual soluble phosphorus.

A similar batch test contained all the inorganic components (including carbonate and magnesium) of the substrate and the EDTA. These results are presented in Figure 19. There was a significant increase

Table 19

Batch Chemical Precipitation Tests

Time (hrs)	Calcium (mM/l)	Phosphorus (mM/l)	Magnesium (mM/l)	Fluoride (mg/l)
0	2.0	0.310	2.0	-
60	2.0	0.310	2.0	-
0	2.0	0.310	2.0	1.0
60	2.0	0.310	2.0	0.85
0 (1)	2.0	0.310	-	-
27	1.5	0.071	-	-
0 (2)	2.0	0.310	-	1.0
27	1.4	0.047	-	<0.1

(1) - Light scattering noted at 10 hours
(2) - Light scattering noted at 14 hours

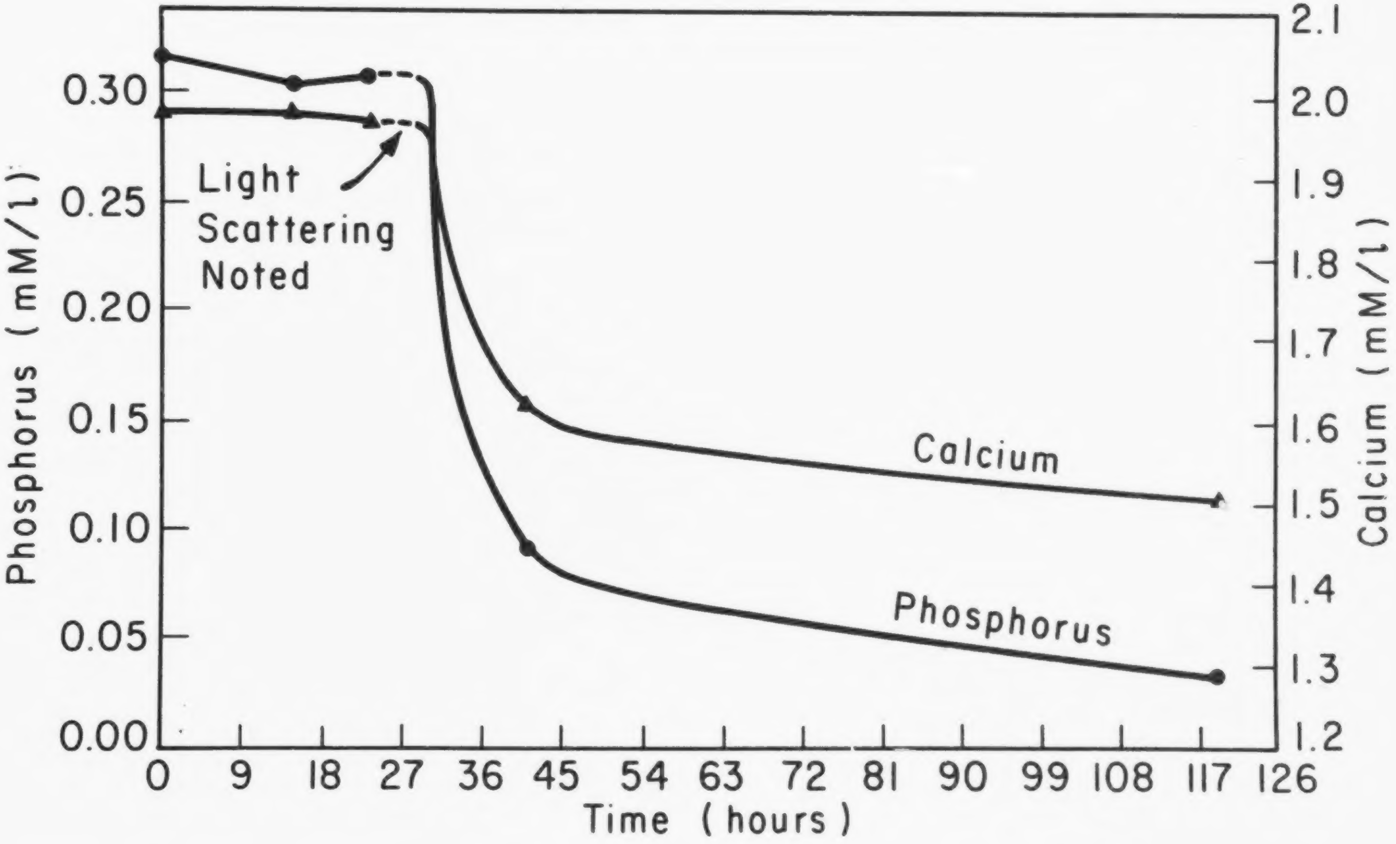

Figure 18. Batch Chemical Precipitation Tests; 2 mM Calcium, 0.33 mM Phosphorus, and Inorganic Components of Substrate C Less Magnesium.

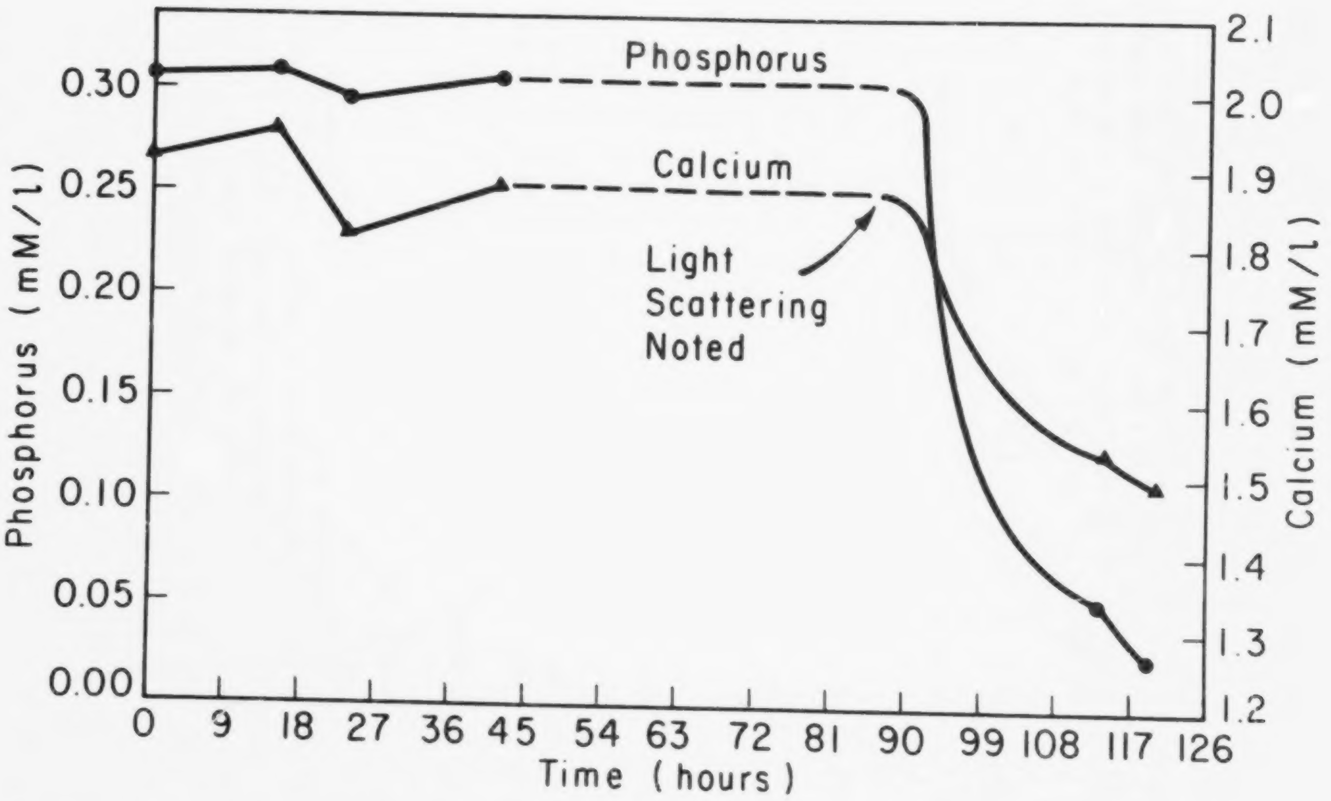

Figure 19. Batch Chemical Precipitation Tests; 2.0 mM Calcium, 0.33 mM Phosphorus, and all Inorganic Components of Substrate C.

in the induction period, almost 3 times that of the test with the inorganic components containing carbonate but not magnesium. Although the induction period was much greater, the residual soluble calcium and phosphorus were not significantly different from the previous tests, and the effect of the inorganic components such as carbonate and magnesium at these concentrations again appeared to be kinetic.

After 114 hours, 20 mg/l of hydroxyapatite was added to the solution. A comparison of results obtained before and six hours after the apatite spike does not indicate significant additional phosphorus removal, although the lowest soluble phosphorus residuals did occur in this test.

Effect of Organic Substrate Components: The effect of the presence of the organic components of the substrate was investigated in the next batch series. The organic components of the substrate were added at a concentration of 50 mg/l COD. (The theoretical carbon concentration in the aeration basin of a completely mixed continuous flow activated sludge system is the effluent carbon concentration. Assuming 90 percent COD removal of a substrate with a COD concentration of 500 mg/l during later experiments, 50 mg/l COD would duplicate the expected carbon concentration during the biological experiments.) The effects of the inorganic components of the substrate were investigated as done in the previous batch tests. Table 20 presents the results after 120 hours.

A calcium-phosphorus precipitation occurred in all the tests. As noted in Table 20, the pH of the batch tests containing all the components of the substrate and all the components less magnesium initially went to 9. Turbidity was noted in the solutions, but appeared to solubilize when the pH was adjusted to 7.6. Apparently some solid form remained in the colloidal state providing nucleation sites, lowering the residual soluble calcium and phosphorus.

An additional batch series was conducted at varying COD concentrations, 0, 50, 100 and 150 mg/l, in solutions containing 2.0 mM calcium, 0.33 mM phosphorus, and all inorganic components (including carbonate and magnesium) of Substrate C. After 120 hours the solution containing no organic component had a residual soluble phosphorus of 0.05 mM (1.55 mg/l), and the solution with 50 mg/l COD had a residual soluble phosphorus of 0.185 mM (5.75 mg/l). However, the solutions with 100 and 150 mg/l COD did not have any measureable precipitation.

Table 20

Batch Chemical Precipitation Tests; 2.0 mM/l Calcium, 0.33 mM/l Phosphorus, and 50 mg/l COD of Organic Components of Substrate C

Additional Components	Initial pH	Final pH	Calcium			Phosphorus		
			Initial mM/l	Final mM/l	Precipitated mM/l	Initial mM/l	Final mM/l	Precipitated mM/l
	7.6	6.4	1.95	1.72	0.30	0.319	0.196	0.111
Magnesium (1)	7.6	6.4	1.97	1.71	0.26	0.306	0.190	0.116
Inorganic Components less magnesium	7.6 (2)	6.2	1.98	1.47	0.51	0.316	0.061	0.255
All Components of Substrate	7.6 (2)	6.15	1.98	1.59	0.39	0.309	0.061	0.248

(1) - 0.8 mM/l; Ca:Mg of 2.5

(2) - Increased to pH 9 during initial pH adjustment. Immediately readjusted to 7.6.

Synopsis

The objective of these tests was to define operating conditions for biological systems with respect to concentrations of calcium and phosphorus and pH representative of actual wastewaters such that a chemical precipitation of phosphorus with calcium would occur. The results from field tests of activated sludge plants reporting unusually high sludge phosphorus contents and laboratory studies of calcium-phosphorus systems led to the investigation of a system with 2.0 mM (80 mg/l) calcium and 0.33 mM (10 mg/l) phosphorus at pH 7.6.

Significant precipitation of calcium and phosphorus occurred at these concentrations in a calcium-phosphorus only system. The addition of 0.8 mM magnesium (a Ca:Mg ratio of 2.5) did not exert a kinetic effect, nor change the residual soluble phosphorus after 41 hours. The residual soluble phosphorus after 41 hours was 0.071 mM (2.21 mg/l) in the calcium-phosphorus system and 0.079 mM (2.45 mg/l) in the system with a Ca:Mg ratio of 2.5.

A calcium- phosphorus system with 2.0 mM magnesium (a Ca:Mg ratio of 1) did not exhibit any measureable precipitation after 60 hours.

A batch test of a calcium-phosphorus system with 1 mg/l fluoride induced precipitation with a lower residual soluble phosphorus, 0.047 mM (1.47 mg/l) than a similar test with no fluoride, 0.071 mM (2.21 mg/l): a difference of 0.024 mM (0.74 mg/l) after 27 hours. Less than 0.1 mg/l fluoride remained in solution indicating that fluoride did enter into the reaction.

A similar system with 1 mg/l fluoride and 2.0 mM magnesium did not induce precipitation. Fluoride apparently did not induce the formation of fluoroapatite.

Batch tests with the inorganic components of the substrate (including carbonate) indicated that only a kinetic effect was exerted in solutions at a Ca:Mg ratio of 2.5, but no phosphorus precipitated at a Ca:Mg ratio of 1.

The addition of organic components of the substrate at a concentration of 50 mg/l COD increased the residual soluble phosphorus, but significant precipitation still occurred. A concentration of 100 mg/l did prevent precipitation.

With the exception of the microorganisms in the activated sludge all the components of the completely mixed continuous flow activated sludge units were present in the test solutions in which significant phosphorus precipitation with calcium occurred. Operation under these conditions should induce precipitation in the concurrent chemical and metabolic incorporation experiments.

Calcium Precipitation of Phosphorus in Completely Mixed Continuous Flow Laboratory Activated Sludge Units

With the operating conditions previously determined, phosphorus incorporation in the sludge by chemical precipitation with calcium could be induced or precluded in a synthetic wastewater substrate. Comparison between concurrently operated units, one favoring and the other precluding significant chemical precipitation, would define the sludge phosphorus content incorporated by a chemical removal mechanism. Also, concurrent operation of two additional units favoring chemical precipitation, one with a Ca:Mg ratio of 1 and one with 1 mg/l fluoride in the substrate, was included to evaluate their effect at these concentrations in an activated sludge system.

General Procedures

Four completely mixed continuous flow laboratory activated sludge units were used during the experimental tests. All four test units utilized a phosphorus concentration of 0.41 mM (12.7 mg/l). The increase in yeast extract and additions of Sego to the substrate increased the total phosphorus concentration in the basic substrate. To maintain continuity with previous experimental tests 10 mg/l of orthophosphate also were added to the substrate in this test series, and this increased the initial total phosphorus concentration to 12.7 mg/l. This increase should not have had a significant effect on the calcium-phosphorus system as most of this 2.7 mg/l increase was in combined forms of phosphorus, i.e., growth factors present in the yeast extract and Sego. Again, the composition of the substrate with respect to minor trace metals precluded significant precipitation of phosphorus with these cations (see Chapter 5). The units were:

1) a "control unit" with 1.0 mM (40 mg/l) calcium similar to the continuous flow tests described in Chapter 5;
2) a "normal Ca:Mg ratio unit" with a Ca:Mg ratio of 2.5 containing 2.0 mM (80 mg/l) calcium;

3) a "low Ca:Mg ratio unit" with a Ca:Mg ratio of 1.0 containing 2.0 mM (80 mg/l) calcium; and,

4) a "fluoride unit" with 1 mg/l fluoride added to the substrate, and with a Ca:Mg ratio of 2.5 containing 2.0 mM (80 mg/l) calcium.

Inoculum for these tests was obtained by using activated sludge as taken from the treatment plant. The sludge was screened and elutriated to remove grit and discrete particles. The units were operated for 6 weeks, approximately 4 biological solids retention time, with the control unit substrate. This removed any solid forms of calcium or phosphorus that may have been occluded within the inoculum, and also acclimated the sludge to the substrate. Prior to beginning the experimental tests the sludges from the 4 units were mixed together and then reapportioned, thus assuring a common inoculum that contained no solid forms of calcium or phosphorus and was acclimated to the substrate.

Substrate C provided a carbon concentration of 500 mg/l COD. During the first part of the tests solids were wasted to maintain 1200 mg/l VSS. This required small variations in the quantity wasted daily, particularly during periods of sludge bulking. When no bulking was occurring the quantity wasted varied from 0.7 to 1.0 liter. In the second part of the tests daily sludge wasting was constant, 0.8 liter per day, and the VSS remained within 1200± 200 mg/l. This wasting schedule provided a maximum biological solids retention time of 10 days. An average effluent suspended solids of 20 mg/l would reduce this to approximately 9 days. The organic loading varied between 0.35 and 0.5 pounds COD per pound VSS per day with a hydraulic residence time of 24 hours.

As in previous tests the substrate contained 2.5 mM bicarbonate. This quantity provided a substrate with an alkalinity similar to that reported for activated sludge systems with unexplained high phosphorus removal.

The pH during the tests was to be maintained at 7.6 in all units. The automatic pH control system only had two sets of probes, and pH control for 4 units was required. During the acclimation period the pH was monitored closely and it was determined that manual control of two units was possible. The operating conditions favored a "natural" pH close to 7.6 and very little titrant was used by the automatic system to maintain this value.

In previous experimental tests the percent sludge phosphorus was computed applying a 0.92 recovery factor (see Chapter 4). The presence of inorganic solid phosphorus within the sludge mass as a result of the precipitation of phosphorus with calcium produced sludge phosphorus contents exceeding the normal "metabolic" phosphorus content. There is no way to quantitatively differentiate between the portion of sludge phosphorus content attributable to metabolic incorporation, which would have the recovery factor applied, and that portion due to inorganic solid phosphorus which would not have the recovery factor applied. The normal variation of sludge phosphorus content precluded applying the recovery factor to the sludge phosphorus content of the control unit and using this value for all units. Less relative error would be expected in not applying the recovery factor to any unit, and this was done.

To provide a means of comparing metabolic incorporation (required plus storage as defined in Chapter 5) of phosphorus to another removal mechanism, the phosphorus required to remove 100 pounds of COD, 0.86 pounds of phosphorus, determined by Menar and Jenkins (34) was used. The quantity of phosphorus required, 4.0 mg/l, for a 92 percent reduction of COD in the test substrate is shown on the graph depicting phosphorus removal from the substrate.

Calcium data between days 8 and 22 was not obtained because of equipment malfunction.

Control Unit

During the 39 days of the test, sludge phosphorus content of the control unit varied from a low of 2.0 percent to a high of 3.07 percent on day 29. However, the sludge phosphorus content decreased to 2.8 percent on day 33 and did not exceed 2.8 percent for the remainder of the test.

With the exception of the first week of operation the phosphorus removed from the substrate was within 1 mg/l of the expected 0.86 pounds phosphorus per 100 pounds COD removed. No significant (greater than 1 mg/l) removal of calcium occurred during the test.

Normal Calcium : Magnesium Ratio Unit

Figures 20 and 21 present the results of the test unit with 2 mM/l calcium compared to the control unit. Initially, the units were similar, no unusual sludge phosphorus content was noted. After one week of

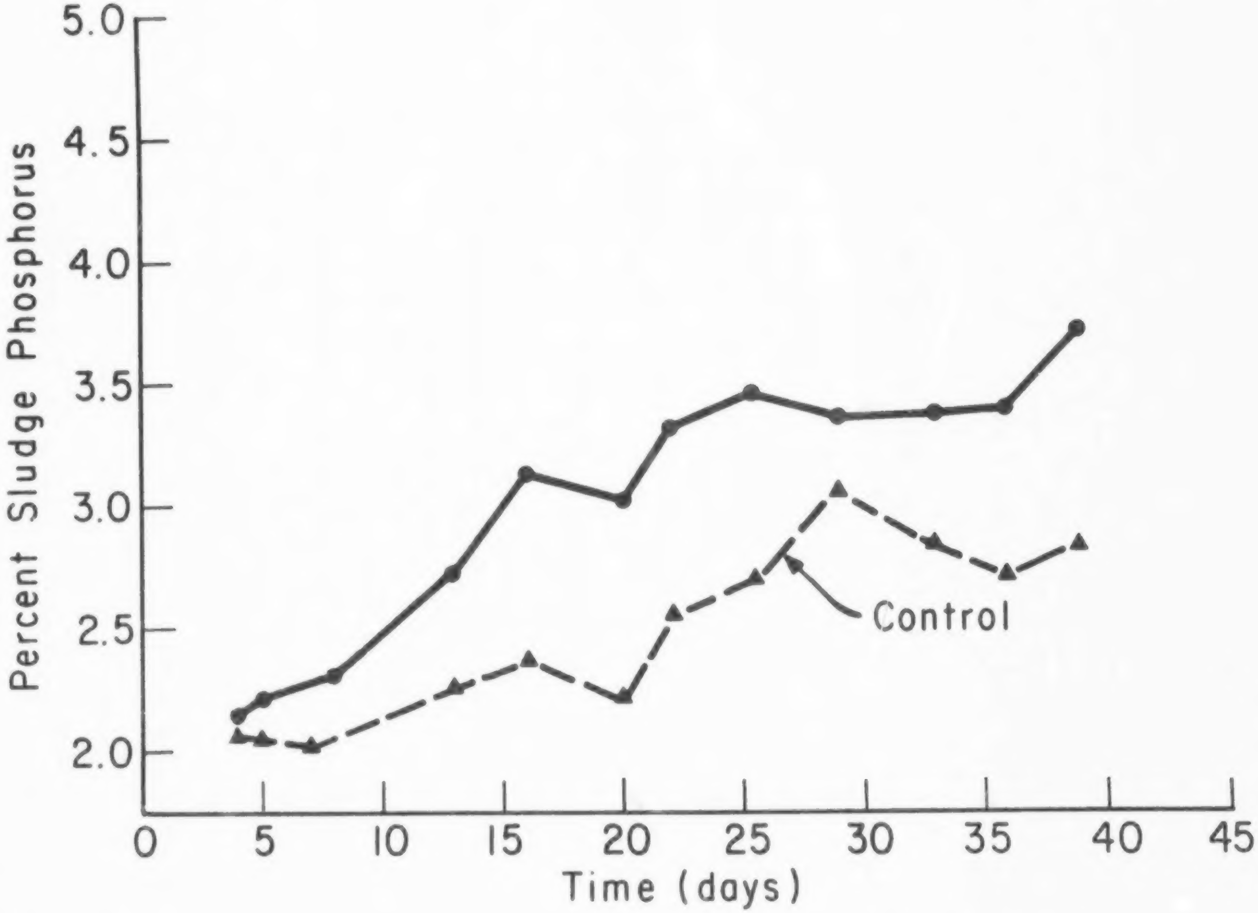

Figure 20. Percent Sludge Phosphorus Content for Six Week Acclimated Laboratory Activated Sludge Unit: Normal Ca:Mg Ratio of 2.5.

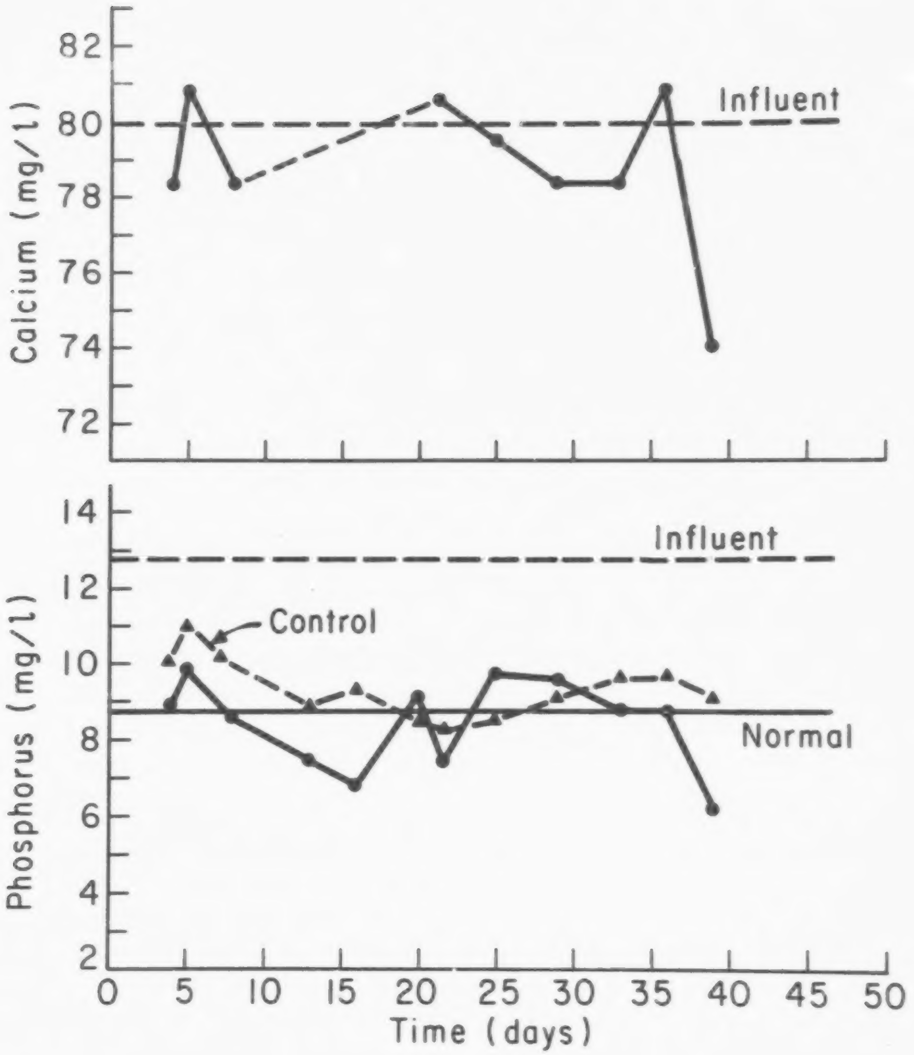

Figure 21. Effluent Calcium and Phosphorus Concentrations for Six Week Acclimated Laboratory Activated Sludge Unit: Normal Ca:Mg Ratio of 2.5.

operation the test unit began to increase in sludge phosphorus content, and this increase was accompanied by increased removal of phosphorus from the substrate. After 16 days a difference of 0.75 percent in the sludge phosphorus content between the test and control units existed.

On day 17 the pH dropped to 7.3, and then increased to 8.4 over a 6 hour period. The pH was slowly readjusted to 7.6 over a 2 hour period. During the third week of operation the sludge in the test unit began to bulk. The bulking lasted for five days.

After 22 days the sludge phosphorus content had increased to 3.4 percent and appeared to be leveling off. The test unit had removed more phosphorus from the substrate than the control except during the period of bulking. Calcium removal was also occurring. After 39 days ($\sim$4 biological solids retention time) the sludge phosphorus content of the test unit increased to 3.7 percent. Approximately 2.5 mg/l of phosphorus in excess of "normal" metabolic requirements was removed from the substrate, which was accompanied by a removal of 6 mg/l (0.15 mM/l) calcium. The sludge phosphorus content of the control unit was 2.8 percent at this time.

Low Calcium : Magnesium Ratio Unit

Figures 22 and 23 compare the results of the low Ca:Mg ratio unit to the control unit. The relationship of this test unit to the control unit is similar to the results obtained in the test unit with the normal substrate Ca:Mg ratio except that a longer lag period occurred. The phosphorus removed from the substrate in the test unit always exceeded that removed by the control unit, although the maximum difference was 1.4 mg/l and was generally less than 1 mg/l.

A comparison of the calcium and phosphorus removed from the substrate in this test does not indicate a relationship similar to the normal Ca:Mg ratio unit. The percent sludge phosphorus appeared to level off at the same time as the normal Ca:Mg ratio unit and the maximum sludge phosphorus content attained after 39 days, 3.7 percent, was the same as the normal Ca:Mg ratio unit. No significant bulking occurred in this unit.

Fluoride Unit

Figures 24 and 25 compare the test unit with 1 mg/l fluoride in the substrate to the control unit. The relationship to the control unit again was similar to the other two test units until the third week. Then, when the other units appeared to be leveling off in percent sludge phosphorus content, the fluoride unit began a steady increase in percent sludge phosphorus content. After 36 days of operation the sludge phosphorus content exceeded 4 percent, and after 39 days was 4.6 percent. This was 1.8 percent more than the control unit. No significant bulking occurred in this unit.

The phosphorus removed from the substrate for the first 22 days was similar to the other test units, except the normal Ca:Mg ratio unit during the period of bulking. As seen in Figure 25 phosphorus removal from the substrate after this time, and particularly during the period of steady increase in percent sludge phosphorus content, began to increase in relation to the other units. Calcium removal occurred at the same time. The quantity removed varied, but appeared to be related to the removal of phosphorus from the substrate. On day 39 the increase of sludge phosphorus content to 4.6 percent was accompanied by both increased phosphorus and calcium removal, approximately 4.3 mg/l (0.14 mM) phosphorus above "normal" metabolic requirements and 7.3 mg/l (0.183 mM) calcium.

During the early period of operation the fluoride concentration in the mixed liquor remained relatively constant at 0.9 mg/l, but did begin to decrease slightly after 29 days. By day 36, 0.25 mg/l fluoride was removed. On day 39, a 0.25 mg/l removal also occurred. Though some fluoride was being removed, it did not exceed 0.25 mg/l at any time.

Synopsis

A six week acclimation period operating under the control unit conditions preceded the actual test period. This assured that any solid forms of phosphorus present in the initial inoculum were removed during sludge wasting, and any subsequent solid forms of phosphorus could be attributed to a precipitation with calcium.

The results of the normal Ca:Mg ratio test unit indicated that a calcium-phosphorus solid had precipitated. The percent sludge phosphorus was higher than the control unit, a maximum of 0.9 percent. No further effect from a low Ca:Mg ratio was observed. The normal and low ratio units differed only in an apparent kinetic effect. The maximum sludge phosphorus content attained by both units was 3.7 percent.

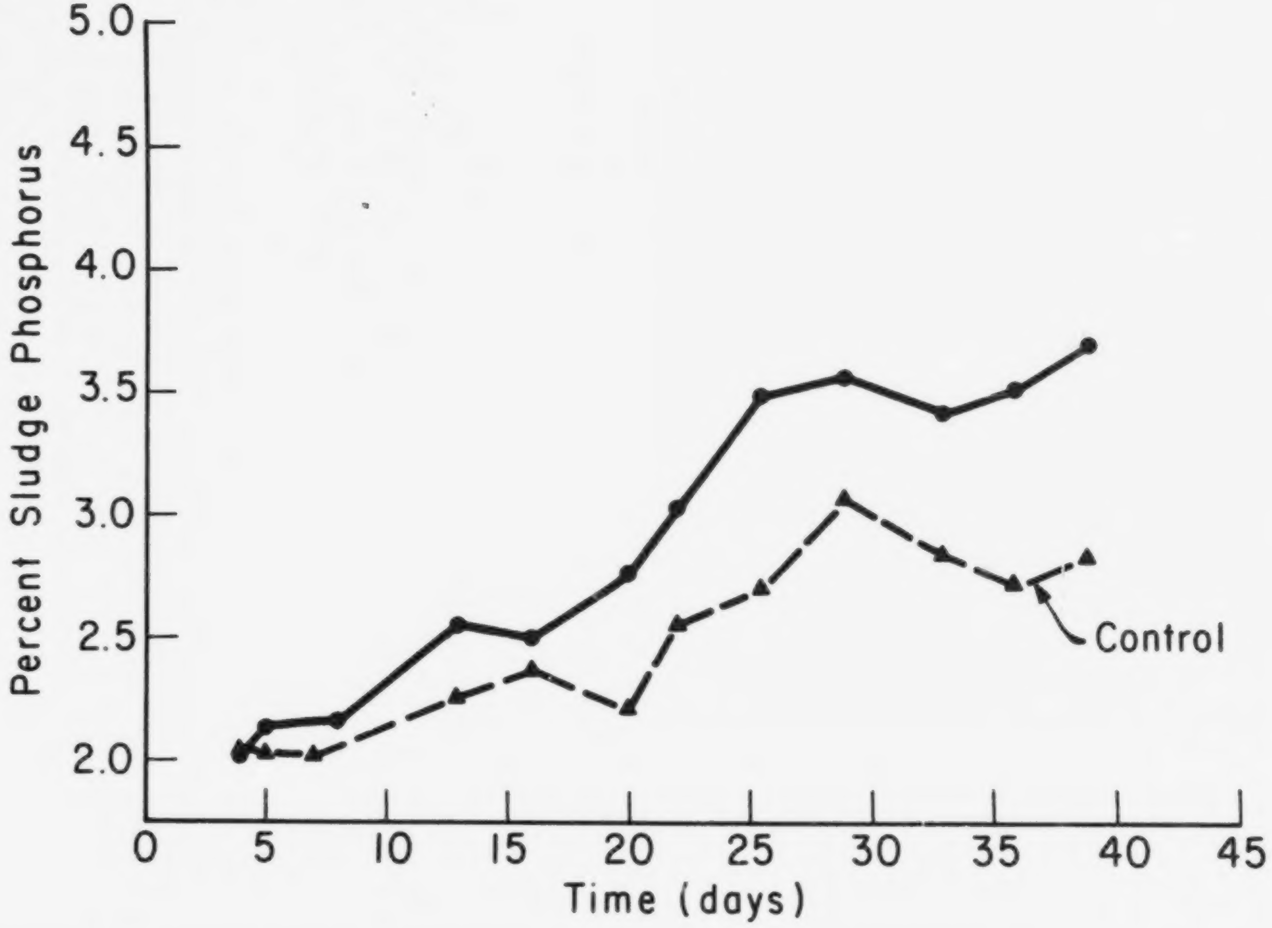

Figure 22. Percent Sludge Phosphorus Content for Six Week Acclimated Laboratory Activated Sludge Unit: Low Ca:Mg Ratio of 1.

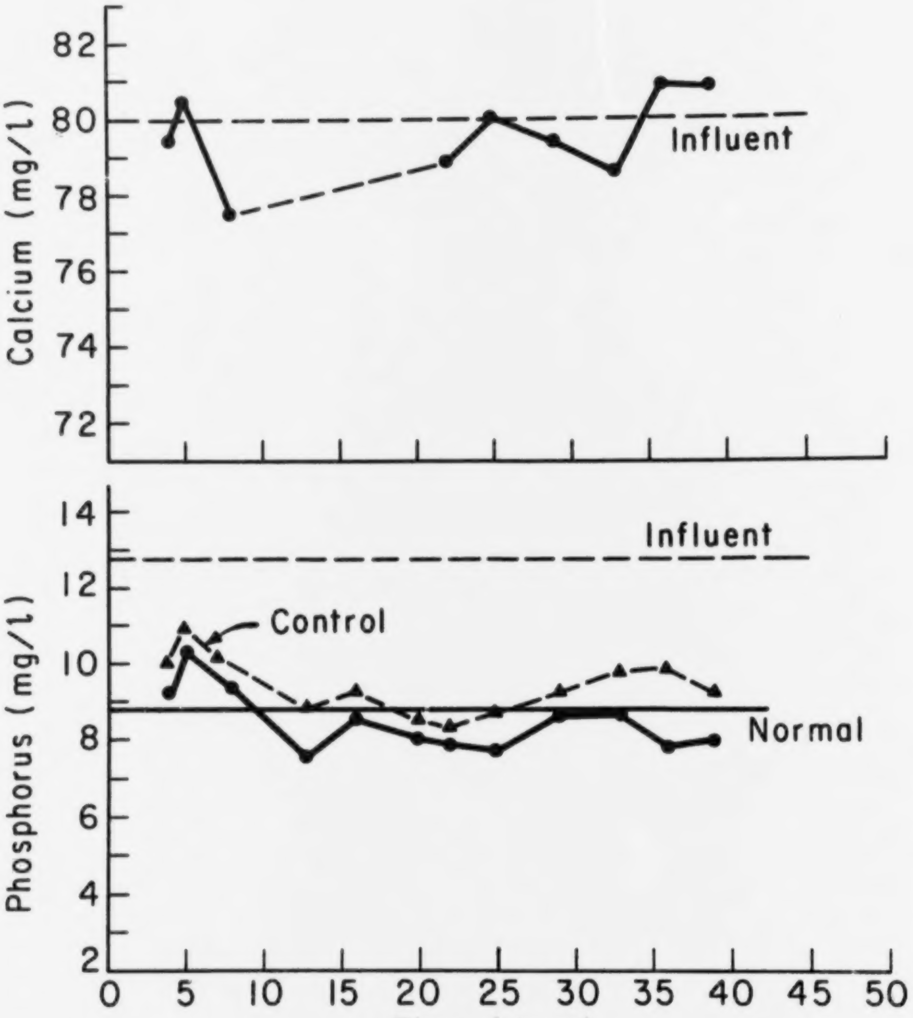

Figure 23. Effluent Calcium and Phosphorus Concentrations for Six Week Acclimated Laboratory Activated Sludge Unit: Low Ca:Mg Ratio of 1.

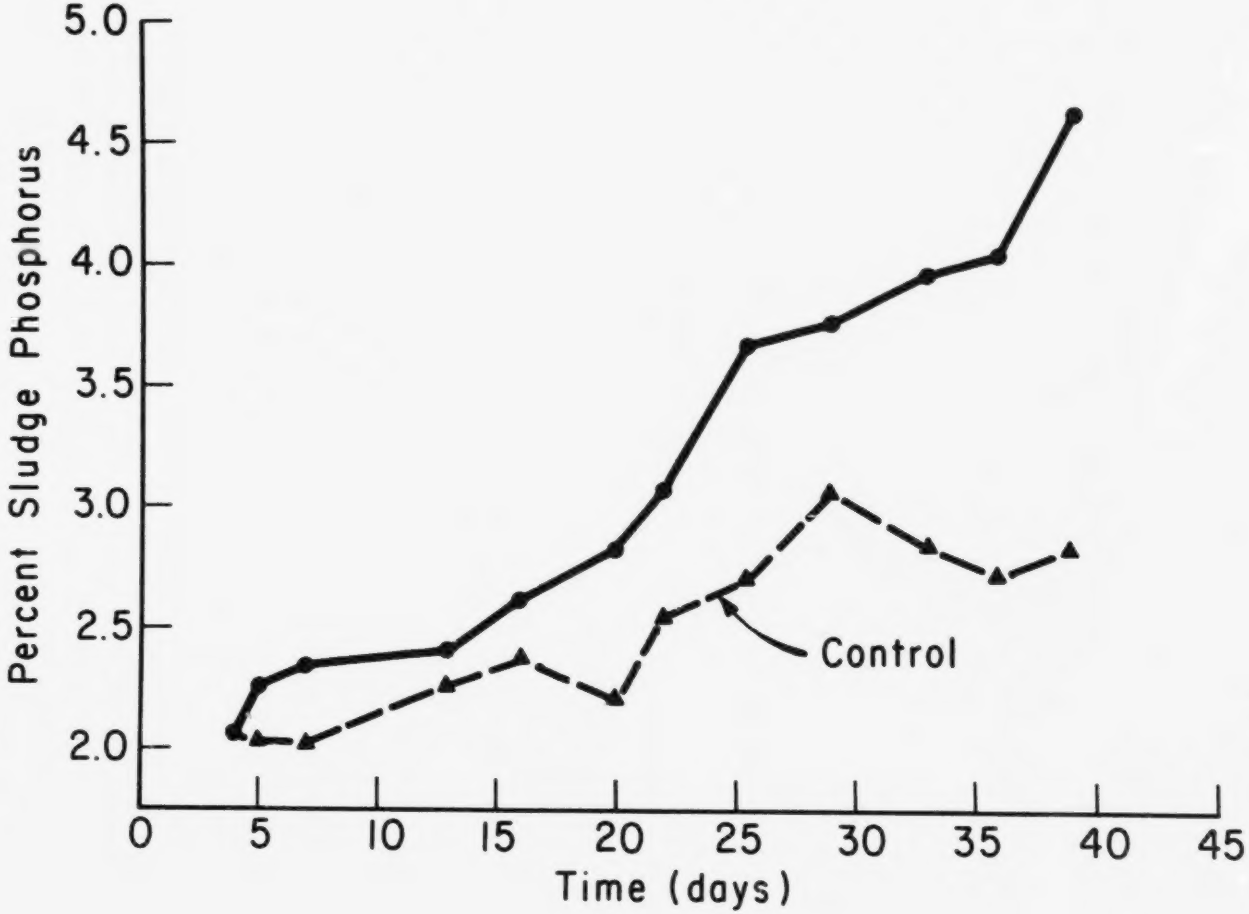

Figure 24. Percent Sludge Phosphorus Content for Six Week Acclimated Laboratory Activated Sludge Unit: Fluoride Unit.

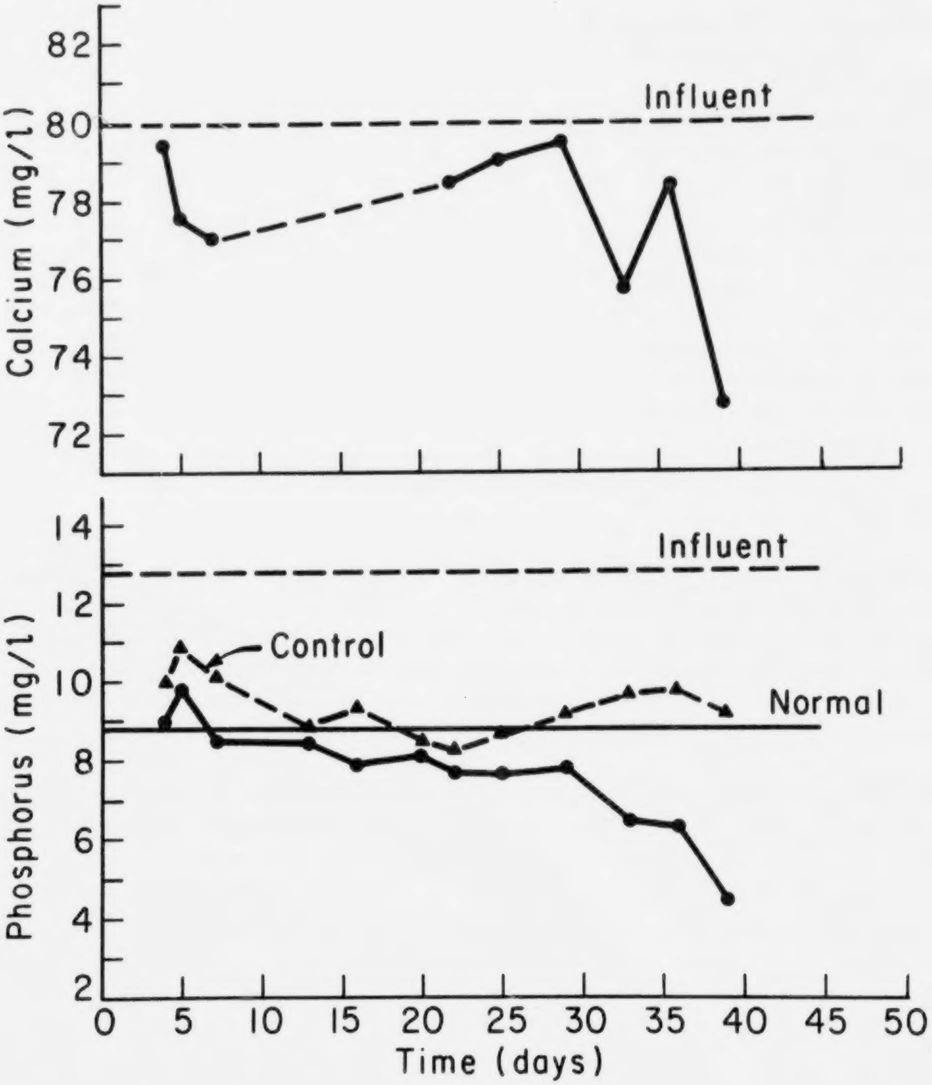

Figure 25. Effluent Calcium and Phosphorus Concentrations for Six Week Laboratory Activated Sludge Unit: Fluoride Unit.

The unit with 1 mg/l fluoride attained the highest sludge phosphorus content, 4.6 percent. The removal of calcium and phosphorus from the substrate attest to the formation of a calcium-phosphorus compound. Fluoride did apparently enter into the reaction with 0.25 mg/l removed from the substrate during this period of high sludge phosphorus content.

Effect of an Induced Upset on Activated Sludge Systems Exhibiting Calcium-Phosphorus Precipitation

During the previous test period a calcium-phosphorus precipitation was induced in the three test units after four biological solids retention time. At this time the three test units were subjected to a temporary upset in operating conditions such that chemical precipitation of phosphorus with calcium was inhibited. This change was in the form of the pH reversal used during the metabolic incorporation of phosphorus with controlled chemical precipitation study in Chapter 5. The pH of the three test units was decreased to 7, held at this pH for 4 hours, then slowly readjusted to pH 7.6 over an additional four hour period. No other operational changes occurred and the general procedures remained the same.

Control Unit

The control unit was not subjected to the upset. Normal operation of this unit occurred until approximately day 60. At that time severe bulking occurred, and the results became somewhat erratic. However, the percent sludge phosphorus remained between 2.2 and 2.7. Phosphorus removal was erratic and less than the "normal" required phosphorus for metabolic uses during this test period.

The reduction in phosphorus removal from the substrate occurred during the periods when the sludge phosphorus content declined. The reduction in sludge phosphorus content was accompanied by a release of phosphorus, and this release increased the phosphorus available for metabolic uses. Calcium effluent was comparable to influent at all times.

Normal Calcium: Magnesium Ratio Unit

At the end of the previous test period, day 39, the normal Ca:Mg ratio unit had 3.7 percent sludge phosphorus content, an effluent phosphorus concentration of 6 mg/l, and an effluent calcium concentration of 74 mg/l. On day 48, 9 days after the pH adjustment the percent sludge phosphorus had decreased to 3.4 percent, effluent phosphorus had increased to 8.1 mg/l, and the calcium effluent had increased to 78 mg/l. Figures 26 and 27 present the results of this test unit.

On day 55, a slight decrease to 3.3 percent sludge phosphorus content occurred. Phosphorus removal from the substrate had increased slightly, but the effluent calcium had increased to approximately the influent concentration. The unit was still removing more phosphorus than required for "normal" metabolic requirement; however, recovery from the upset had not occured in 16 days.

Because the major difference between the 3 previous test units had occurred with the addition of fluoride to the substrate, fluoride was added at this time to this unit and the results will be discussed subsequently.

Low Calcium : Magnesium Ratio Unit

Figures 28 and 29 present the results of the low Ca:Mg ratio unit. At the end of the previous test period, this unit had 3.7 percent sludge phosphorus content, an effluent phosphorus concentration of 7.95 mg/l, and an effluent calcium concentration of 80.8 mg/l. On day 48, the sludge phosphorus content had decreased to 3.25 percent, effluent phosphorus concentration had increased to 8.6 mg/l, and the effluent calcium had increased slightly to 81.6 mg/l. No significant difference in sludge phosphorus content was noted between the different Ca:Mg ratio units with no apparent recovery occurring in this unit.

(At this time the concentration of magnesium was reduced in this unit to the normal ratio to acclimate the unit for an experiment with the addition of a solid calcium-phosphorus spike similar to that done in Chapter 5. The results will be discussed in a subsequent section.)

Fluoride Unit

Figures 30 and 31 present the results of the unit with 1 mg/l of fluoride in the substrate. This unit had the highest sludge phosphorus content, 4.6 percent, and the maximum removals of calcium and phosphorus, 7.3 mg/l and 8.3 mg/l respectively, in the previous test. The phosphorus removed from the substrate was over twice that required for "normal" metabolic uptake.

This unit had the largest reduction in sludge phosphorus content, a decrease of almost 1 percent, after the upset. This unit experienced

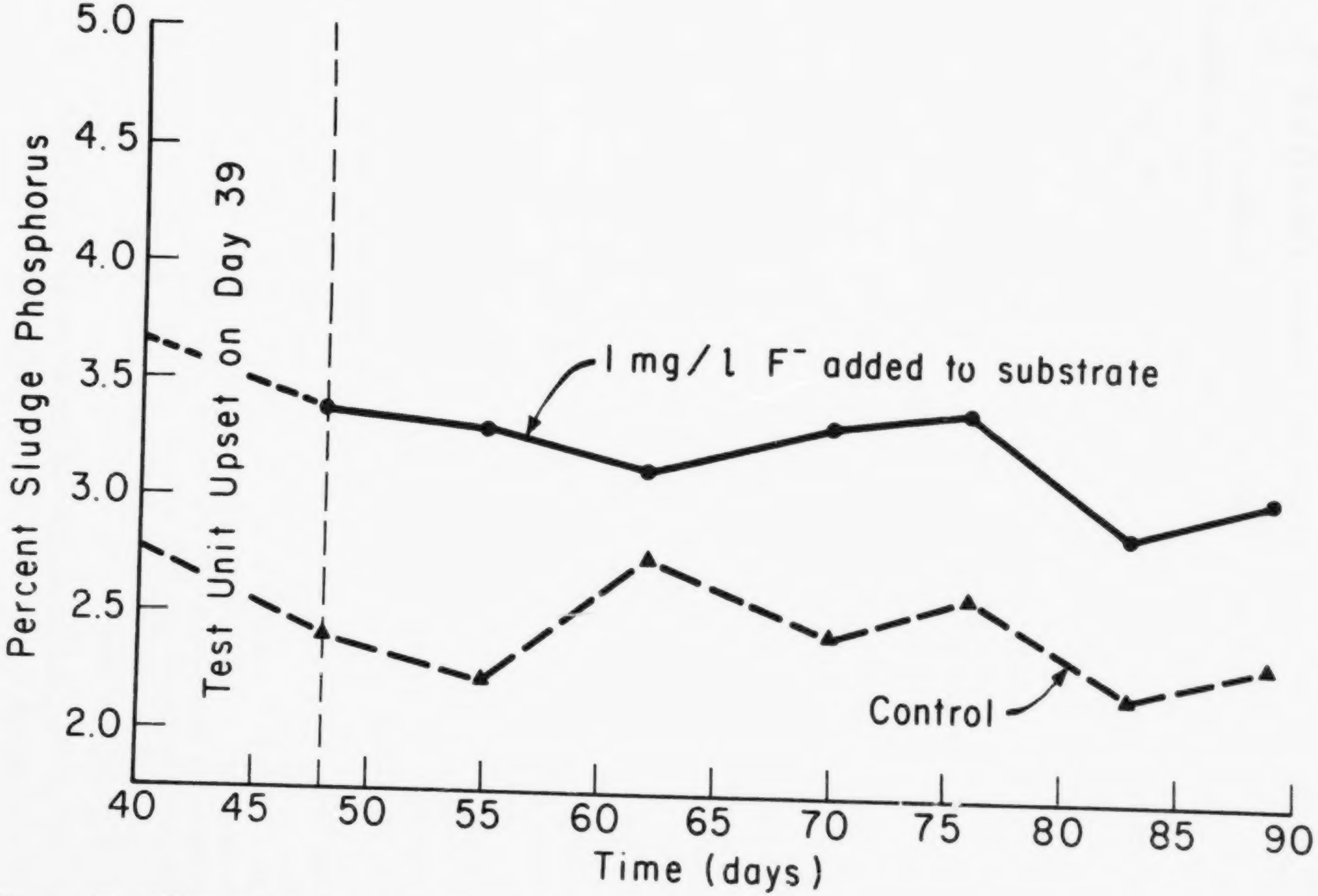

Figure 26. Effect of pH Upset on Percent Sludge Phosphorus in Laboratory Activated Sludge Unit: Ca:Mg Ratio of 2.5.

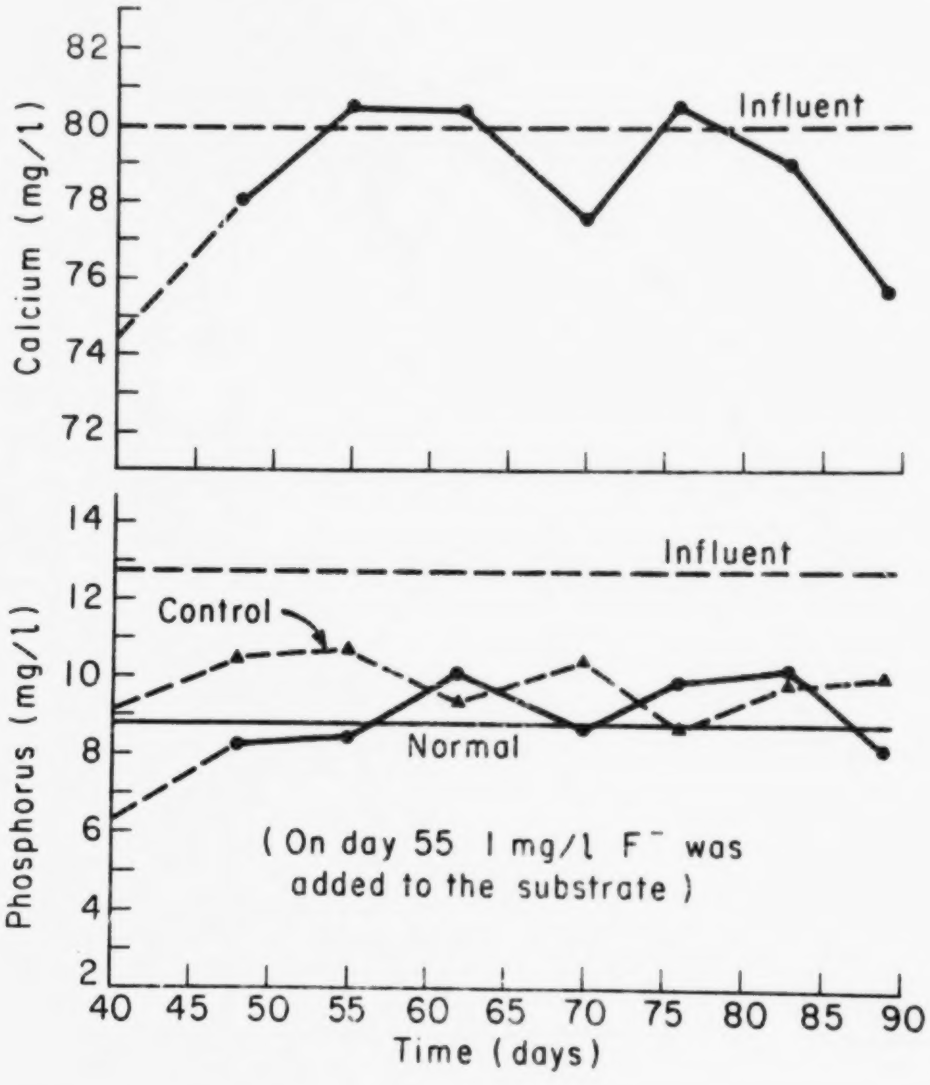

Figure 27. Effect of pH Upset on Laboratory Activated Sludge Unit: Ca:Mg Ratio of 2.5.

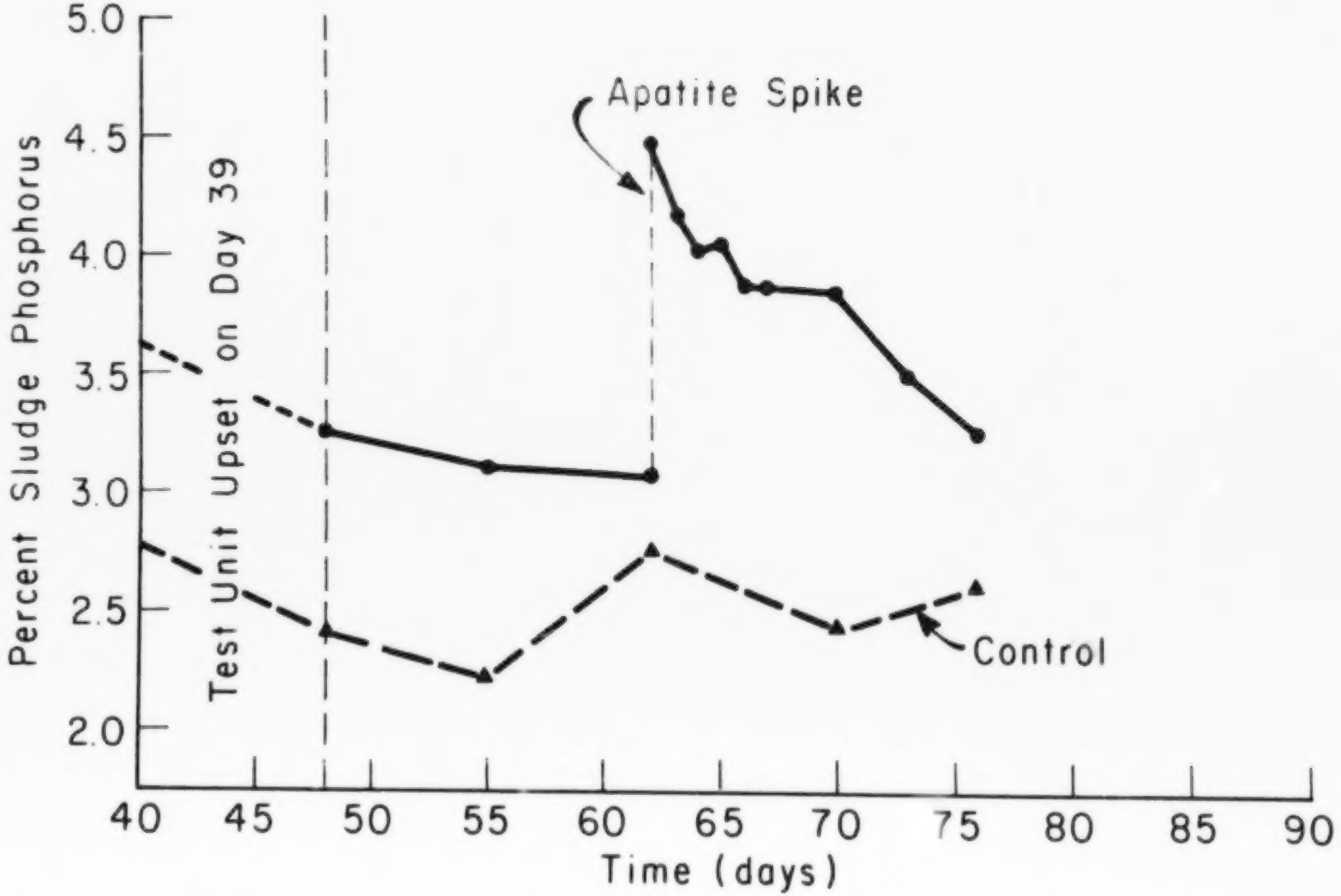

Figure 28. Effect of pH Upset and Addition of Apatite Spike on Percent Sludge Phosphorus in Laboratory Activated Sludge Unit: Low Ca:Mg Ratio of 1; Increased to 2.5 for Apatite Spike.

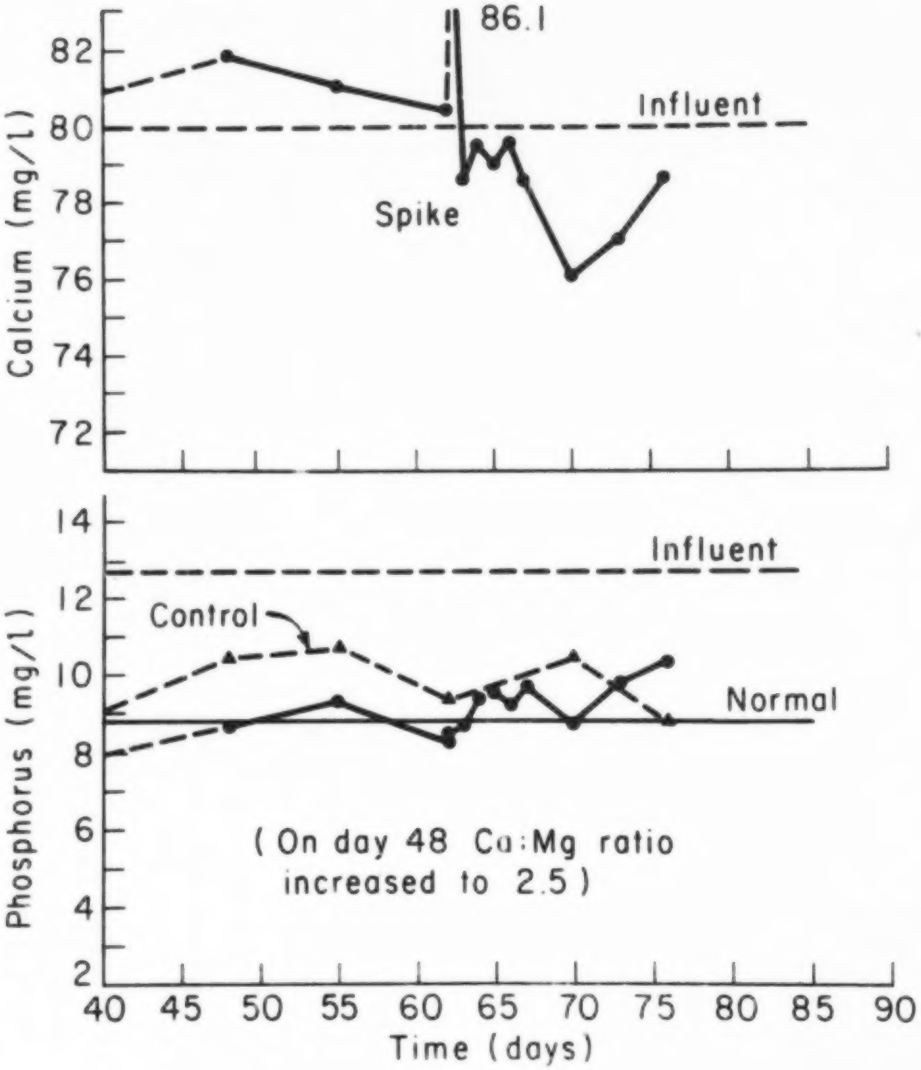

Figure 29. Effect of pH Upset and Addition of Apatite on Laboratory Activated Sludge Unit: Low Ca:Mg Ratio of 1; Increased to 2.5 for Apatite Spike.

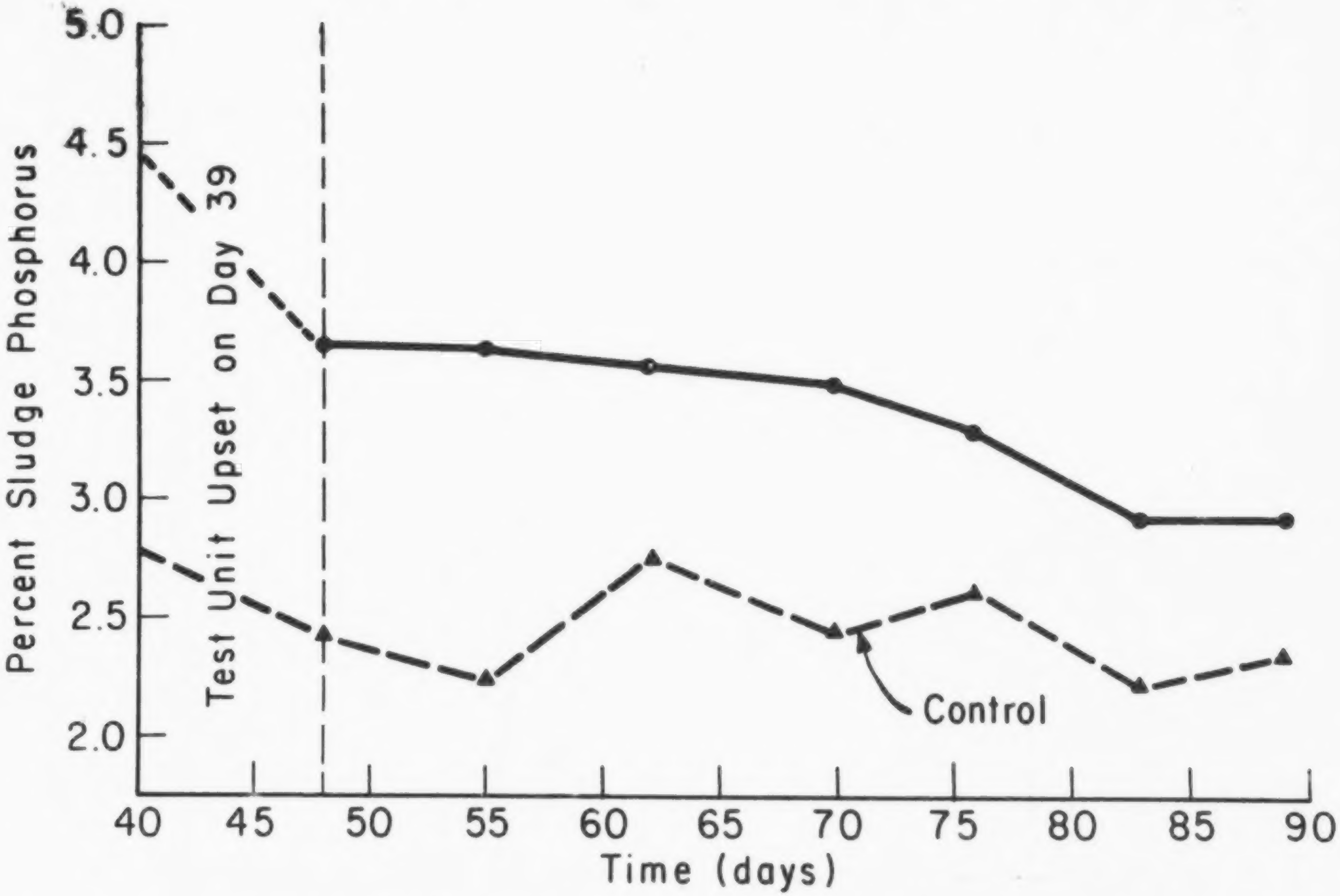

Figure 30. Effect of pH Upset on Percent Sludge Phosphorus in Laboratory Activated Sludge Unit: Fluoride Unit.

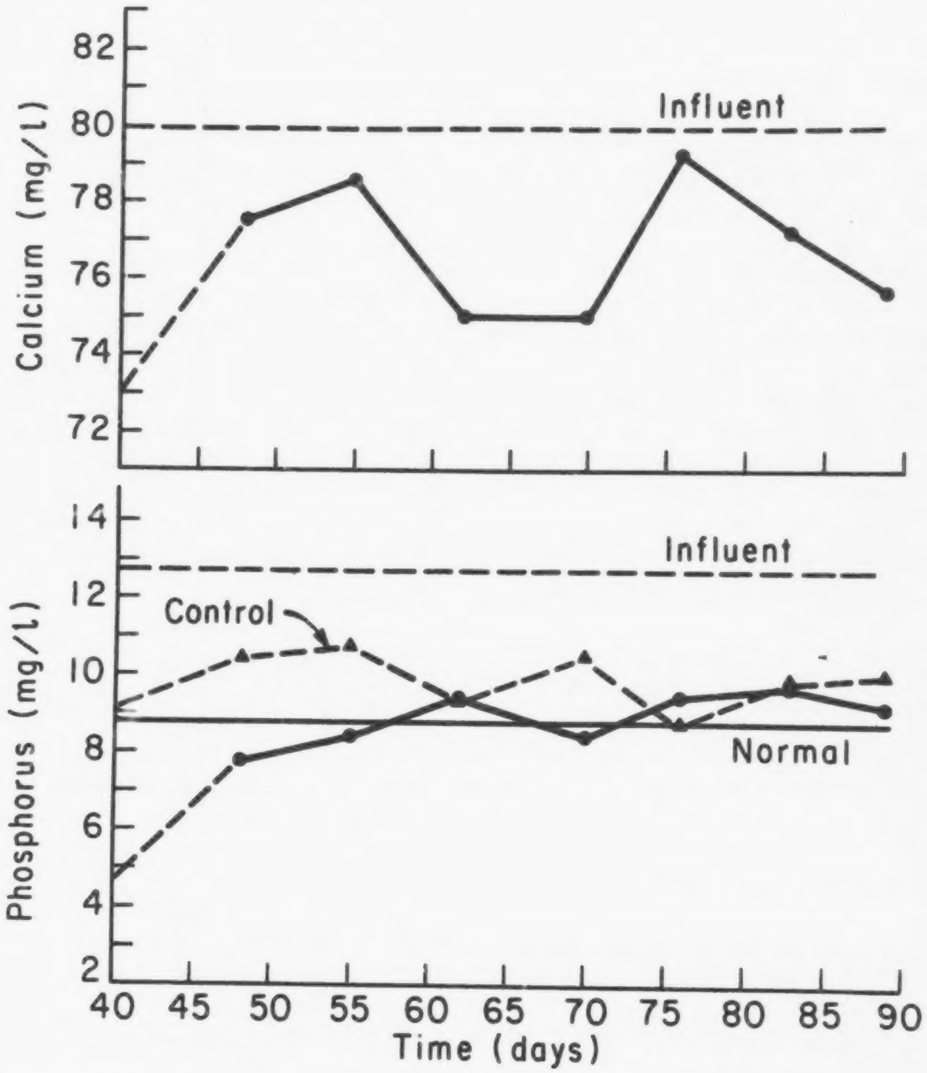

Figure 31. Effect of pH Upset on Laboratory Activated Sludge Unit: Fluoride Unit.

a slow decrease in sludge phosphorus content, from 3.65 on day 48 to a low of 2.9 on day 83. During this period calcium was removed from the influent, but this was not matched by phosphorus removals significantly different from "normal" metabolic requirements. This unit experienced severe bulking between day 65 and 80. At the completion of the test run on day 89, this unit had a sludge phosphorus content of 2.9 percent.

The residual soluble fluoride did not decrease below 0.9 mg/l following the upset.

On day 55 fluoride (1 mg/l) was added to the substrate of the normal Ca:Mg ratio unit. This gave two units with fluoride and provided duplicate units for observation.

On day 62, the sludge phosphorus content in this new fluoride unit decreased to 3.1 percent which was matched by an increase in effluent phosphorus. This unit experienced severe bulking after day 65. On day 70 the sludge phosphorus again increased to 3.3 percent and the effluent phosphorus decreased slightly. From this time on the results were erratic; however, the percent sludge phosphorus content exceeded that of the control unit by at least 0.6 percent during the last 20 days of operation and was 0.75 percent greater at the completion of the test run on day 89. Calcium was being removed from the influent, but phosphorus uptake was at or above the "normal" metabolic requirement during this period.

The residual soluble fluoride also did not decrease below 0.9 mg/l for this new fluoride unit.

The removals of calcium, phosphorus and fluoride and the percent sludge phosphorus content were approximately the same in the two fluoride units from day 70 to the end of the test period.

Effect of Added Solid Hydroxyapatite

On day 62, the solid spike, hydroxyapatite (tri-basic calcium phosphate) was added to the previous low Ca:Mg ratio unit. Prior to the spike the unit had 3.05 percent sludge phosphorus, and after the spike, 80 mg/l $Ca_{10}(PO_4)_6(OH)_2$, the percent sludge phosphorus content should have increased to 4.55 percent.

Fifteen minutes after the addition of the spike the sludge phosphorus content had increased to 4.5 percent and the effluent phosphorus increased to 8.45 mg/l, an increase of only 0.2 mg/l. The effluent calcium increased to 86.1 mg/l, an increase of 5.8 mg/l. Although the calcium effluent concentration increased, it was apparent that the spike did not significantly solubilize. See Figures 26 and 27.

Daily analyses were performed for the next 5 days. During this time, no unusual calcium or phosphorus release occurred to the effluent and the percent sludge phosphorus decreased as a function of sludge wasting. On day 70, the sludge phosphorus content appeared to stabilize near 3.85 percent and this was accompanied by an increase in the removals of calcium and phosphorus from the substrate. However, further sludge phosphorus reduction occurred on days 73 and 76 with decreased removals of calcium and phosphorus from the substrate.

The solid spike was not solubilized, but it also did not induce increased phosphorus incorporation by the sludge. This experimental unit was terminated at this time.

Synopsis

The temporary upset of the three test units caused a significant reduction in the sludge phosphorus contents. In the fluoride unit the sludge phosphorus content decreased almost 1 percent and in the other units decreased approximately 0.4 percent. Recovery did not occur in any unit after 2 biological solids retention times. However, in the test units the sludge phosphorus content did continue to exceed that of the control unit by 0.8 percent.

The effect of fluoride that occurred during the previous test period ceased. No significant fluoride removal from the substrate occurred for the remainder of the test period, 50 days.

Fluoride was added to the substrate of the normal Ca:Mg ratio unit on day 55, giving two test units with fluoride for comparison. By day 70 the results of both units were almost identical and continued in this manner for the duration of the test. Neither unit removed any significant fluoride from the substrate.

The effect of a solid spike was observed by the addition of hydroxyapatite to a test unit. The addition of this solid did not induce additional calcium-phosphorus removal from the substrate. The added sludge phosphorus content from the spike decreased during sludge wasting and was no longer evident after one biological solids retention time.

The control unit was not subjected to the upset and continued to operate normally. The sludge phosphorus content varied between 2.2 and 2.85 percent.

The test units did maintain a sludge phosphorus content above the normal range for activated sludge, i.e. greater than 3 percent, during most of the test period. A low of 2.9 percent occurred in the fluoride unit on day 89, but the control unit sludge phosphorus content was 2.3 percent at this time. Although higher sludge phosphorus contents were maintained by the three test units compared with control, the much higher sludge phosphorus contents attained during the earlier tests were not regained after the upset.

Summary

A synthetic wastewater substrate was developed that induced significant chemical precipitation of phosphorus with calcium. A concurrent chemical and metabolic incorporation of phosphorus test series was conducted to evaluate the magnitude of this chemical removal mechanism.

No significant phosphorus was precipitated in the control unit. The substrate of this unit contained calcium and phosphorus concentrations similar to the substrates used during the metabolic incorporation of phosphorus precluding chemical precipitation, but at a pH of 7.6. These results substantiate the results obtained during that study with respect to precluding significant phosphorus precipitation at pH 7.0.

A chemical precipitation of phosphorus did occur at pH 7.6 and concentrations of 2 mM calcium and 0.33 mM phosphorus. The quantity of phosphorus precipitated in the continuous flow experiments was less than indicated by batch chemical precipitation tests containing all components of the substrate. However, the absence of microorganisms during the batch chemical precipitation tests limited evaluation of all components of the activated sludge system. The presence of the microorganisms probably can exert an effect on the formation of calcium-phosphorus solids, precluding ordered crystals, and a possibility exists that metabolic by-products can act as crystal poisons. This was substantiated in part by the batch experiments showing that increasing COD concentrations inhibited phosphorus removal.

A Ca:Mg ratio of 1 did not exert a long term effect on percent sludge phosphorus content. Both the normal Ca:Mg ratio of 2.5 and the Ca:Mg ratio of 1 units attained 3.7 percent sludge phosphorus content after 39 days of operation.

The test unit with 1 mg/l fluoride obtained the highest sludge phosphorus content, 4.6 percent. The removal of fluoride, 0.25 mg/l, from the substrate indicated that fluoride did enter the reaction. However, after the upset of the units the presence of fluoride in the substrate was not significant. Two test units had 1 mg/l fluoride during a 34 day test period, and no significant fluoride removal occurred in either unit.

The addition of a solid form of calcium-phosphorus, hydroxyapatite, did not induce additional phosphorus incorporation in the sludge mass.

Although phosphorus was precipitated by calcium during these tests at pH 7.6, it is apparent that this removal mechanism is sensitive to upset.

SECTION VII

DISCUSSION

The objectives of this research were to determine the mechanisms of high phosphorus removal from some wastewaters with subsequent high sludge phosphorus contents. The research scope was limited to an evaluation of two commonly proposed mechanisms: metabolic phosphorus incorporation (both required and "luxury uptake") by activated sludge cultures, and calcium-phosphorus precipitation. In addition an alkaline phosphatase bioassay was evaluated to define "luxury uptake" of phosphorus.

Metabolic Phosphorus Incorporation

The results of short term (less than one biological solids retention time) completely mixed continuous flow laboratory activated sludge systems operated under saturated dissolved oxygen conditions with substrates that precluded chemical precipitation are compared to the results of Hattingh (16) in Figure 32. Hattingh reported an influent COD:phosphorus ratio of 250:1 (100:0.4) as "optimum"; i.e., the concentration of influent phosphorus such that phosphorus just begins to appear in the effluent. Sawyer (60) reported an influent optimum BOD:phosphorus ratio of 150:1 (~COD:phosphorus ratio of 220:1). The value obtained in the present research, 220:1 (100:0.45), agrees with these past results.

The percent sludge phosphorus content reported by Hattingh and that obtained in this research at this "optimum" ratio was approximately 1.5 to 1.6 percent. At influent COD:phosphorus ratios less than 220:1 phosphorus appeared in the effluent of all systems studied in this investigation. The percent sludge phosphorus content varied from 1.6 to 3.07 when significant chemical precipitation was precluded for a number of different tests. Phosphorus removal varied from 0.5 to 1.0 pounds phosphorus removal per 100 pounds COD removed. These results compare quite closely with past investigators (3,15, 16, 20).

"Luxury uptake" of phosphorus can be defined as that not necessary for normal growth, i.e., a constant 85 to 90 percent carbon removal. Applying this definition as Borchardt and Azad (17) described for specific algal cultures (see Figure 1) any sludge phosphorus content

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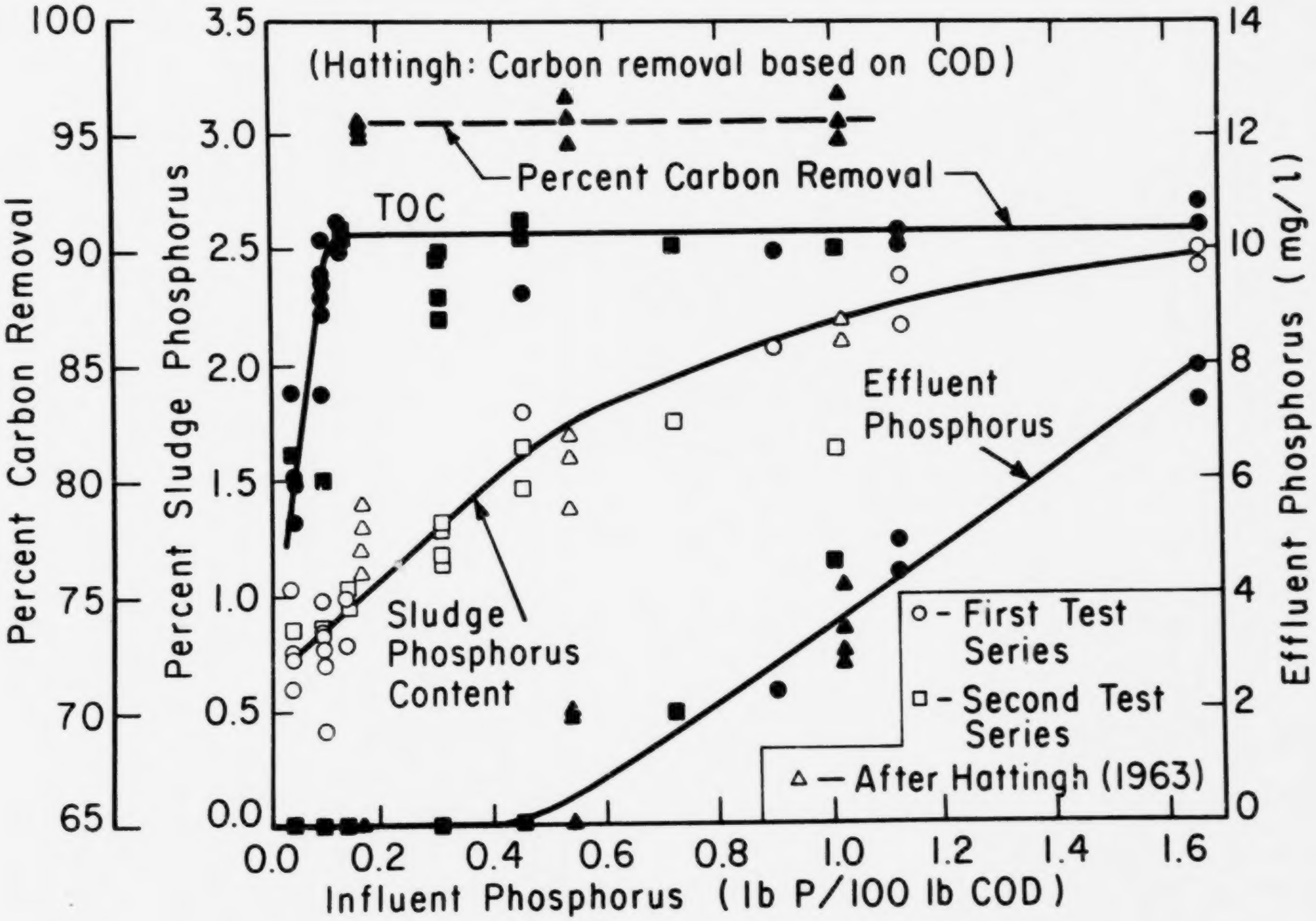

Figure 32. Influent Phosphorus, Effluent Phosphorus, Percent Sludge Phosphorus Content, and Percent Carbon Removal for Laboratory Completely Mixed Continuous Flow Activated Sludge Units.

beyond 1 percent would be termed "luxury uptake." The range between 1 and 1.6 percent sludge phosphorus content would be the storage zone for activated sludge; all phosphorus present in the influent would be incorporated in the sludge. A saturation zone as described by Borchardt and Azad for specific algae cannot be finitely defined for activated sludge because of the heterogeneous nature of the biomass, but will vary from 1.6 to approximately 3 percent.

Referring to the conditions depicted in Figure 32, relatively high alkaline phosphatase activity was exhibited in the phosphorus limiting condition below 1 percent sludge phosphorus content. A transition occurred with gradually reduced enzymatic activity exhibited as the sludge phosphorus content increased towards 1.6 percent. Above 1.6 percent the alkaline phosphatase activity remained relatively low and constant. The enzymatic bioassay did define the general range of storage between 1 and 1.6 percent sludge phosphorus content. However, above 1.6 percent sludge phosphorus content the enzymatic activity did not define the upper limit of the saturation zone.

The maximum sludge phosphorus content obtained in any test defining the limits of metabolic incorporation of phosphorus was approximately 3 percent. The minimum sludge phosphorus content tests, and also substantiated by the alkaline phosphatase bioassay, indicate that a "luxury uptake" of phosphorus by activated sludge is not the exception, but the rule.

In evaluating these results from an engineering aspect a primary consideration is that above 1.6 percent sludge phosphorus content phosphorus appears in the effluent in the absence of any chemical precipitation. In general almost all activated sludge systems treating municipal wastes operate in this area. However, if operation could be achieved below 1.6 percent sludge phosphorus content, a major benefit would occur: no significant phosphorus in the effluent. The addition of carbon as shown by Sawyer (1) to obtain the "optimum" carbon to phosphorus ratio depicted in Figure 32 is not feasible.

Without regard to the influent carbon to phosphorus ratio a feasible operation could occur by reducing the phosphorus content of the return sludge to some value below 1.6 percent. The minimum value would be approximately 1 percent, that required for normal growth. Neglecting new growth an increase in sludge phosphorus content from 1 to 1.6 percent in an activated sludge system with 2000 mg/l volatile suspended solids represents the incorporation of 12 mg/l phosphorus. This quantity of phosphorus is typical of the influent concentration found in many municipal wastewaters.

Such a scheme was first proposed by Levin and Shapiro (8). The stripping of phosphorus from activated sludge can be accomplished using a period of anaerobiosis or slightly acidifying the sludge. The sludge is returned to the system after sufficient phosphorus is stripped and the concentrated supernatant receives further treatment prior to ultimate disposal. Additionally, digester supernatant must not be returned to the activated sludge system. Although significant phosphorus removal is possible using this scheme, two areas of concern appear. Initially, the sludge must be stripped of phosphorus. The use of short time anaerobic stripping does not appear feasible. The City of Trenton, Michigan (61), investigated the use of anaerobic stripping and found that significant phosphorus release required up to 2 days. During the course of the present research a similar time period was required to reduce the phosphorus content to levels below 1.5 percent (see Section IV). Adequate short time stripping can be achieved using acidification, but imposes both a chemical requirement and the need for a suitable pH control and monitoring system to avoid toxic pH conditions.

A "normal" activated sludge with 2.6 percent sludge phosphorus content could be reduced to 2 percent; however, a return to 2.6 percent with no significant phosphorus appearing in the effluent would not be assured. The results of this research and past investigators indicate that beyond 1.6 percent sludge phosphorus content phosphorus will appear in the effluent. This imposes an upper limit of 1.6 percent for such a scheme.

Consideration of these two areas would be necessary in evaluating possible use of this phosphorus removal scheme. The cost of additional treatment for stripping with adequate safeguards and the range of phosphorus removal possible would dictate a comparison with other phosphorus removal methods for a specific wastewater. Additionally, the effect of a phosphorus stripping scheme on normal operating characteristics of activated sludge systems, with particular reference to adequate and rapid solids-liquid separation, is not known. No general conclusion as to the effectiveness or practicality of this removal method can be made.

Calcium-Phosphorus Precipitation

Using six week acclimated completely mixed continuous flow laboratory activated sludge units with a synthetic wastewater substrate representative of actual municipal wastewaters, a sludge phosphorus content of 3.7 percent was obtained. The substrate composition restricted chemical precipitation to calcium-phosphorus forms and contained 0.41 mM (12.7 mg/l) phosphorus with 2.5 mM (150 mg/l) bicarbonate. The pH of the continuous flow units was controlled at 7.6. The sludge phosphorus content of the unit with 2.0 mM (80 mg/l) calcium was 0.9 percent higher than an identical unit with only 1.0 mM (40 mg/l) calcium which restricted significant calcium-phosphorus precipitation.

A comparison of the continuous flow activated sludge units with a "normal" Ca:Mg ratio of 2.5 and a low Ca:Mg ratio of 1 revealed no significant differences after 39 days of operation. Three weeks of operation were required to produce a significant calcium-phosphorus precipitation in the unit with a Ca:Mg ratio of 1, but this was only 5 to 7 days more than required for the unit with a Ca:Mg ratio of 2.5. Both units had sludge phosphorus contents of 3.7 percent after 39 days.

Ferguson, _et al_. (37) reported that an uncertainty exists as to the effect of low Ca:Mg ratios on dilute calcium-phosphorus system. The effect of magnesium is hypothesized to be either mainly kinetic, (a delay in initiation of the precipitation) or to have a significant effect on the type of calcium-phosphorus solid that is formed, (β tri-calcium phosphate vice an apatite). The results of batch chemical precipitation tests for several calcium-phosphrous systems are presented in Figure 33. A calcium-phosphorus system with 2.5 mM magnesium (Ca:Mg of 2.5) did not show any kinetic effect, nor indicate a solid form with a different Ca:P ratio as a result of the presence of magnesium. However, a calcium-phosphorus system with a Ca:Mg ratio of 1 did not exhibit any measureable precipitation after 60 hours.

The addition of the inorganic components of Substrate C, less magnesium, to a calcium phosphorus system did exert a kinetic effect, tripling the time required for precipitation. This substrate contained 2.5 mM/l bicarbonate. This result is similar to that reported by Ferguson, _et al_. for a calcium-phosphorus system with 3.8 mM

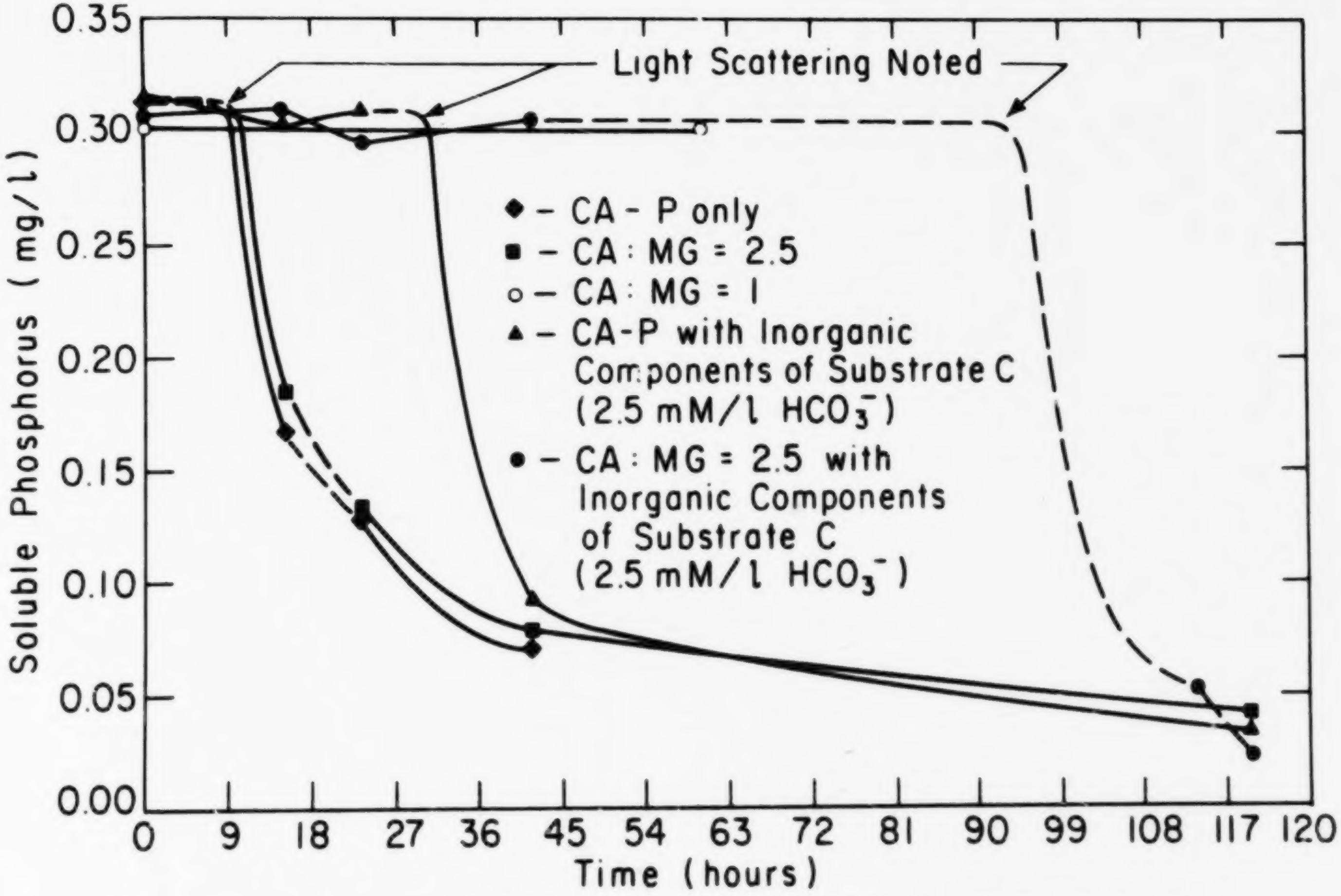

Figure 33. Qualitative Summary of Phosphorus Precipitation found in Batch Tests with 2.0 mM Calcium.

bicarbonate. Addition of magnesium, Ca:Mg ratio of 2.5, to a test solution with the inorganic components of the substrate (including 2.5 mM bicarbonate) did cause a kinetic effect, extending the time required for precipitation by a factor of 3. No significant effect was noted in residual soluble phosphorus between test solutions.

Menar and Jenkins (34) reported the results of an extensive pilot-plant study using a wastewater with a Ca:Mg ratio of 0.9. Phosphorus incorporation in the activated sludge in excess of normal metabolic requirements was obtained; sludge phosphorus contents up to a maximum of 6.8 percent were obtained, but a pH in excess of 8.0 was required. A continuous flow activated sludge unit in the present research with a Ca:Mg ratio of 1 exhibited phosphorus incorporation in the sludge in excess of normal metabolic requirements with a maximum sludge phosphorus content of 3.7 percent. Although the sludge phosphorus content of this research is significantly less than reported by Menar and Jenkins, this was obtained at pH 7.6 and produced a lower effluent phosphorus concentration.

Table 21 compares the two influents. A major difference in operating conditions was alkalinity, 2.5 mM bicarbonate in this research and 405 mg/l as $CaCO_3$ ($\sim$ 8.0 mM bicarbonate) in the wastewater used by Menar and Jenkins. A comparison of the calcium and magnesium concentrations to the total hardness does not indicate the presence of other significant cations.

Thus, it is indicated that magnesium exerts only a kinetic effect in dilute calcium-phosphorus systems, and alkalinity probably affects both the magnitude and kinetics of the precipitation of calcium phosphorus forms.

Table 21

Comparison of Sewage from San Ramon, California
and Synthetic Wastewater Substrate

Constituent	San Ramon (1) (mg/l)	Synthetic Wastewater (mg/l)
Calcium	66 (1.65 mM)	80 (2.0 mM)
Magnesium	44 (1.85 mM)	48 (2.0 mM)
Alkalinity as $CaCO_3$	405	125
pH in	7.7	6.9
pH out	>8.0	7.6
Total Hardness as $CaCO_3$	346	-

(1) Mean of analytical data collected during study.

Addition of 1 mg/l fluoride to the substrate with a Ca:Mg ratio of 2.5 and 2.5 mM bicarbonate further increased the sludge phosphorus content to 4.6 percent, 0.9 percent greater than the unit without fluoride, after 39 days of operation. Over twice the required phosphorus for normal metabolic incorporation was removed and the effluent phosphorus decreased to 4.1 mg/l, the lowest obtained in any experiment. As pointed out by Leckie and Stumm (33) a lower residual soluble phosphorus was obtained with fluoride present in trace quantities. The time required for significant precipitation to occur was slightly longer than the unit without fluoride, but the same as the low Ca:Mg unit.

Though calcium was shown to precipitate phosphorus, it was also shown to be sensitive to upset. Sludge phosphorus contents higher than the control unit were exhibited after a pH upset, but previous levels of sludge phosphorus content were not regained after 18 days of additional operation. The presence of fluoride did not effect the quantity of phosphorus precipitated after the pH upset and continued operation for 50 days did not indicate any effect from the presence of fluoride. The addition of fluoride did enhance phosphorus removal with calcium, but the failure of fluoride to regain a high sludge phosphorus content after upset reduces the engineering significance of fluoride addition to water supplies.

In further assessing the results, the amount of phosphorus incorporated from chemical precipitation in the completely mixed continuous flow activated sludge systems was less than expected from the batch chemical precipitation tests. During the batch tests there were organic substances present, but no microorganisms. In the presence of 50 mg/l COD there was less phosphorus precipitated than in systems containing only inorganics. Also, the presence of organics at twice the concentration in the activated sludge units (100 mg/l COD) prevented any precipitation after 5 days during the batch tests.

Leckie and Stumm (33) and Yu and Mark (39) offer partial explanation for these results. These investigators reported that organic substances can act as crystal poisons. This effect could be a poisoning of nuclei formed in the early stages of precipitation, thereby preventing growth and restricting the precipitation of a solid form to the formation of nuclei. Also, the possibility exists that a more soluble metastable amorphous form is precipitated and the presence of organics prevents the formation of a less soluble stable form (62). Yu and Mark reported that the presence of blue-green algae, acting as a surface active agent, prohibited the formation of ordered crystals of apatite, and an amorphous form developed. The presence of microorganisms could have exerted an effect, both by their presence, as well as from the by-products of their metabolism which may have added organic substances not found in the substrate that were effective crystal poisons.

An organic poisoning effect could be exhibited in plug flow systems in the following manner. At the inlet end of the aeration tank a high carbon concentration exists which is reduced as the flow proceeds down the tank. When the carbon concentration is reduced sufficiently to alleviate the poisoning effect, calcium-phosphorus precipitation could occur.

As pointed out by Menar and Jenkins (34) in discussing the requirement for high dissolved oxygen postulated as enhancing "luxury uptake" of phosphorus (4,5,8), a dissolved oxygen concentration increase fortuitously coincides with a reduction in dissolved and gaseous CO_2 content. Thus an increase in dissolved oxygen should be accompanied by an increase in pH, thereby favoring a chemical precipitation of phosphorus. This point in the aeration tank also coincides generally with the reduction of organic matter, thereby alleviating any organic poisoning effect which could have inhibited chemical precipitation of phosphorus.

The results implicating an organic poisoning of calcium-phosphorus precipitation indicate a re-evaluation of operating conditions reported to enhance phosphorus removal. The conditions observed at the San Antonio Rilling Plant (4) do show an increase in dissolved oxygen with a rise in pH up to 7.6 - 7.8 occurring at the point where high phosphorus removal begins. However, Milbury, et al. (50) report no correlation between pH level and the point of rapid phosphorus removal, although a pH rise did occur across the plug flow aeration tank. This rise was almost linear with no sharp increases and the effluent varied between 6.8 and 7.3. The dissolved oxygen level also was not related to the rapid phosphorus removal, provided the system was not oxygen limited. Oxygen limiting conditions always were associated with phosphorus releases and an accompanying reduction in organic removal. Though the results of several monitoring runs at Baltimore (28) do show a correlation between an increase in dissolved oxygen and the rapid uptake of phosphorus, several runs did not and were presented to show this lack of correlation.

Table 22 presents two of these monitoring runs with the inclusion of organic removal parameters. These data do implicate the occurrence of an organic poisoning effect; a phosphorus removal was associated with a reduction in soluble organic concentration independent of the dissolved oxygen concentration. The 8/27/69 Control data show a drop in COD and TOC through secondary clarification that was accompanied by a removal of 12 mg/l phosphorus. Additional runs presented to show the lack of correlation between dissolved oxygen content and high phosphorus removal were not as widely separated as these two events, but also show a similar relationship.

The results of Milbury, et al. (30) at Baltimore and the inhibitory effect on calcium-phosphorus precipitation found with increasing organic concentrations in the chemical precipitation batch tests would seem to indicate that an organic poisoning effect does exist.

Further consideration of the results obtained in this research using completely mixed systems, a system not normally associated with high phosphorus removal, lend support to this hypothesis. Menar and Jenkins (34) pointed out that an average lower pH in the aeration tank and the residence time distribution are not favorable to calcium-phosphorus precipitation. In this research a pH of 7.6 was maintained; however, a soluble organic concentration, 30 to 55 mg/l COD, was achieved in the aeration tank: a low soluble organic concentration. Such a low soluble organic concentration would have reduced the poisoning effect allowing a calcium-phosphorus precipitation to occur.

In summary calcium was shown to be capable of precipitating phosphorus in the pH range and concentrations of calcium and phosphorus encountered in municipal wastewaters. However, the additional phosphorus removed and incorporated in the sludge by calcium in these experiments would not account for the high removals and sludge phosphorus contents reported for several activated sludge plants. It is apparent that other cations are involved in chemical precipitation of phosphorus in these systems. Those plants reporting cation concentrations through the system do show sufficient removals of several suspected cations, Fe III, Al III, Ca, Zn, to account for the phosphorus removals obtained when taken together. A single cation is not responsible.

Table 22

Relationship Between Soluble Phosphorus, Dissolved Oxygen and Soluble Organics in an Activated Sludge System (after City of Baltimore)

			Position Along Aeration Tank							
9/17/69 - Test	Primary Effluent	Inlet	1/6	1/3	1/2	2/3	5/6	Exit	Return Sludge	Secondary Effluent
Ortho P, mg P/l	8.7	10.2 ❘	1.3	0.6 ¦	0.3	0.2	0.3	0.2	20.6	0.3
D.O., mg/l	-	0.0 ❘	0.1	1.2 ¦	3.6	5.4	5.6	6.6	-	1.6
Soluble BOD, mg/l	210	120.0 ❘	60.0	28.0 ¦	17.0	14.0	13.0	11.0	-	-
Soluble COD, mg/l	240	80.0 ❘	65.0	55.0 ¦	45.0	50.0	45.0	45.0	90.0	50.0
pH	6.5	6.8 ❘	6.9	6.9 ¦	6.9	6.9	7.0	7.0	6.6	7.2
3/27/69 - Control										
Ortho P, mg P/l	4.8	50.8	14.1 ¦	13.9	13.7	-	-	12.5 ❘	78.0	0.5
D.O., mg/l	-	0.0	- ¦	-	6.0	-	-	4.9 ❘	-	1.3
Soluble COD mg/l	130	70.0	60.0 ¦	60.0	50.0	55	40	45.0 ❘	-	35.0
Soluble TOC mg/l	110	65.0	60.0 ¦	60.0	50.0	50	50	50.0 ❘	-	25.0
pH	6.4	6.8	- ¦	-	6.7	-	-	6.7 ❘	6.7	6.8

Note: "❘" designates maximum P removal and "¦" designates maximum D.O. increase.

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SECTION X

APPENDICES

Appendix

A Calcium-Phosphorus Solids . . . .
    Table A-1: Calcium-Phosphorus Solids . . . . . .

B Substrates . . . .
    Table B-1: Substrate A . . . .
    Table B-2: Substrate B . . . .
    Table B-3: Substrate C . . . .
    Table B-4: Trace Elements . . . .

C Analytical Method for Phosphorus Determination .

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Table A-1

Calcium-Phosporus Solids

Chemical Formula	Common Name	Solubility Product pK_{sp} (298° K)
$Ca(H_2PO_4)_2$	monocalcium phosphate	1.14
$Ca\ HPO_4$	dicalcium phosphate	6.90
$Ca\ HPO_4 \cdot 2H_2O$	dicalcium phosphate dihydrate	6.60
$Ca_{10}(PO_4)_6(OH)_2$	hydroxyapatite	115.6
$Ca_{10}(PO_4)_6(F_2)$	fluoroapatite	118.0
$Ca_4H(PO_4)_3 \cdot 3H_2O$	octacalcium phosphate	46.9
$\beta\text{-}Ca_3(PO_4)_2$	beta-tricalcium phosphate	27.0

Table B-1

Substrate A

Element	Concentration (mg/l)	Added as
Carbon	330 (TOC)	* Multi-Carbon Source
Nitrogen	30	NH_4Cl
Potassium	20	KCl
Magnesium	18	$MgCl_2 \cdot 6H_2O$
Sulfur	10	Na_2SO_4
Calcium	6	$CaCl_2 \cdot 2H_2O$
Iron	0.4	$FeCl_3$
Bicarbonate	150	$NaHCO_3$
EDTA	50	$Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$

Trace Elements - 1 ml for each liter of substrate (see Table B-4)

* Multi-Carbon Source

Compound	Concentration - mg/l
Glucose	111
Sucrose	167
Lactose	190
Sodium Citrate	21
Succinic Acid	139
Yeast Extract	62
Peptone	75

Phosphorus - added as KH_2PO_4

Table B-2

Substrate B

Element	Concentration (mg/l)	Added as
Carbon	130 (TOC)	* Multi-Carbon Source
Nitrogen	15	NH_4Cl
Potassium	20	KCl
Magnesium	18	$MgCl_2 \cdot 6H_2O$
Sulfur	10	Na_2SO_4
Iron	0.4	$FeCl_3$
Bicarbonate	150	$NaHCO_3$
EDTA	50	$Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$

Trace Elements - 1 ml for each liter of substrate (see Table B-4)

* Multi-Carbon Source

Compound	Concentration - mg/l
Glucose	44
Sucrose	65
Lactose	75
Sodium Citrate	8.5
Succinic Acid	55
Yeast Extract	2.5
Peptone	30

Phosphorus - added as KH_2PO_4
Calcium - added as $CaCl_2$

Table B-3

Substrate C

Element	Concentration (mg/l)	Added as
Carbon	500 (COD)	* Multi-Carbon Source
Nitrogen	50	NH_4Cl
Potassium	35	KCl
Sulfur	15	Na_2SO_4
Iron	0.4	$FeCl_3$
Bicarbonate	150	$NaHCO_3$
EDTA	50	$Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$

Trace Elements - 1 ml for each liter of substrate (see Table B-4)

*Multi-Carbon Source

Compound	Concentration - mg/l
Glucose	290
Yeast Extract	72
Sego (1)	0.5 (ml/l)

(1) Product of Pet Milk Company

Phosphorus - added as KH_2PO_4
Calcium - added as $CaCl_2$
Magnesium - added as $MgCl_2$
Fluoride - added as NaF

Table B-4

Trace Elements

Element	Concentration (g/1)	Added as
Boron	2.00	H_3BO_3
Zinc	2.00	$ZnSO_4 \cdot 7H_2O$
Manganese	0.40	$MnCl_2 \cdot 4H_2O$
Molybdenum	0.47	MoO_3
Copper	0.40	$CuSO_4 \cdot 5H_2O$
Cobalt	0.10	$Co(NO_3)_2 \cdot 6H_2O$

APPENDIX C

Analytical Method for Phosphorus Determination

The analytical procedures used for total phosphorus determination are described below. Variations from procedures listed in FWPCA Methods for Chemical Analysis of Water and Wastes (55) are shown in parentheses.

Reagents

1. Sulfuric acid solution, 5N: Dilute 70 ml of concentrated H_2SO_4 with distilled water to 500 ml.
2. Potassium antimonyl tartrate solution: Weigh 1.3715 g. $K(SbO)\ C_4H_4O_6 \cdot 1/2\ H_2O$, dissolve in 400 ml distilled water in 500 ml volumetric flask, dilute to volume. Store in glass-stoppered bottle.
3. Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6\ Mo_7O_{24} \cdot {}^4H_2O$ in 500 ml distilled water. Store in plastic bottle at 4° C.
4. Ascorbic acid, 0.1 M: Dissolve 1.76 g. of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if stored at 4°C.
5. Combined reagent: Mix the above reagents in the following proportions for 100 ml of the mixed reagent: 50 ml of 5 N H_2SO_4, 5 ml of potassium antimonyl tartrate solution, 15 ml of ammonium molybdate solution, and 30 ml of ascorbic acid solution. Mix after addition of each reagent.
6. Strong-acid solution: Slowly add 310 ml conc. H_2SO_4 to 600 ml distilled water. When cool, dilute to 1 liter.
7. Potassium persulfate. (ammonium persulfate.)

Procedures

1. Add 1 ml of strong-acid solution to 50 ml of sample in a 250 ml Erlenmeyer flask.

2. Add 0.8 gram of potassium persulfate. (0.4 gram ammonium persulfate.)

3. Autoclave for 1 hour (30 minutes) at 121°C and 15-20 psi.

4. Add phenolphthalein and adjust sample to pink with 1 N H_2SO_4. Bring back to colorless with one drop strong-acid solution, and allow to cool.

5. Transfer to a 100 ml volumetric flask, bring to volume with distilled water.

6. Add 8 ml of combined reagent to sample and mix thoroughly. After a minimum of ten minutes, but no longer than thirty minutes, measure the color absorbance of each sample at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution.

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